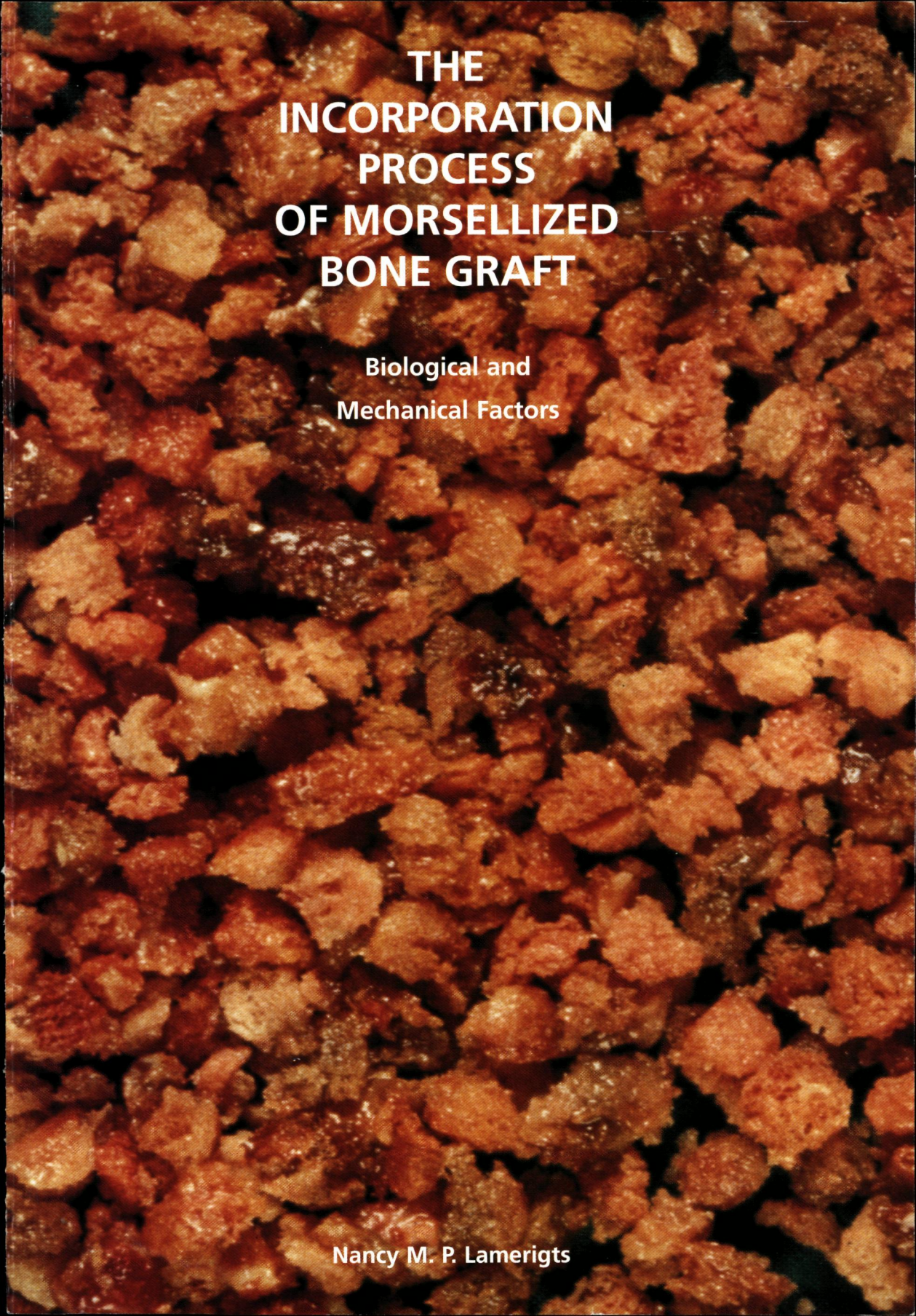
THE
INCORPORATION
PROCESS
OF MORSELLIZED
BONE GRAFT
Biological and
Mechanical Factors
Nancy M. P. Lamerigts

THE INCORPORATION PROCESS OF MORSELLIZED BONE GRAFT

Biological and Mechanical Factors

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG.

Lamerigts, Nancy Maria Petra.

The incorporation process of morsellized bone graft
Biological and mechanical factors.
N. M. P. Lamerigts.
Proefschrift Katholieke Universiteit Nijmegen - met literatuur opgave
en samenvatting in het Nederlands.

ISBN 90-9011628-1
Uitgegeven door Nancy M. P. Lamerigts

Lay-out and printed by Feenstra Design/Advertising, Grootebroek, The Netherlands

The research and the publication of this investigation have been supported by

Howmedica
International.

THE INCORPORATION PROCESS OF MORSELLIZED BONE GRAFT

Biological and Mechanical Factors

een wetenschappelijke proeve op het gebied van de Medische Wetenschappen

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Katholieke Universiteit Nijmegen,
volgens besluit van het College van Decanen in het
openbaar te verdedigen op donderdag 11 juni 1998
des namiddags om 1 30 uur precies

door

NANCY MARIA PETRA LAMERIGTS

geboren op 5 maart 1968
te Beegden

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CONTENTS

5

CHAPTER

GENERAL INTRODUCTION.

Bone grafts represent one of the earliest devised reconstructive approaches in the musculoskeletal system and are practised in most of the commonly used orthopaedic procedures Bone grafts are used to induce fracture healing in non-unions and spinefusion, to replace traumatic bone loss due to tumour resection or to reconstruct deformed joints or failed joint replacements with bone loss Various morphological types and sizes of bone graft are available, like cortical bone graft, cortico-cancellous structural bone graft and cancellous morsellized bone graft

The quality of bone grafts, i e to serve its function for a specific purpose, depends both on its morphological properties and the preservation and processing procedures Preservation and processing procedures, like deep-freezing, freeze drying, demineralization, sterilization by gamma radiation or ethylene oxide and addition of growth factors affect the properties of the graft concerning its capacity to provide for active bone formation, to act as a substrate for bone formation and to exert mechanical stability In general, the fresh frozen grafts appear to have superior supportive qualities, whereas demineralized bone is, due to its sponge-structure, only applicable in non-load bearing regions or in combination with mineralized bone graft In situations of inferior bone healing, the osteoinductive properties of demineralized bone or additional growth factors could be advantageous The choice of a specific type of bone graft therefore may determine the clinical success of a bone graft application

The bone graft incorporation process

The incorporation process of a bone graft, whether it is an autograft or an allograft, seems to be very similar to fracture healing [7,12] A haematoma is formed due to the operation-trauma, resulting in a non-specific inflammatory response to the necrotic bone tissue and necrotic marrow In a second stage, fibrovascular tissue invades the graft Between the bone graft and the host bone connections are formed so that the bone graft is attached to the host bone This process is called *consolidation* Osteoclasts are transported to the inflammatory side through the new formed bloodvessels In a third stage, parts of the dead bone graft are resorbed by osteoclasts, whereafter new bone is apposited on the remaining graft The graft remnants serve as a trellis for the new bone and this is called *osteoconduction*, i e the facilitation and support of bone ingrowth The ingrowing fibrovascular tissue contains primitive non-differentiated mesenchymal cells that are able to differentiate into osteoblasts In imitation of the knowledge of fracture healing, it is assumed that the graft incorporation process is also controlled by growth factors These could be supplied through the fibrovascular inflammatory tissue and released by the dead bone graft during resorption The stimulatory activity of the local growth factors on the primitive mesenchymal cells to differentiate into bone is called *osteoinduction* In the final stages of the graft incorporation, the dead graft-new bone mixture is further remodelled into a bone structure, which vitality is directly related to the proceeding of the incorporation

Factors involved in the bone graft incorporation process

The size and architecture of the bone graft influence the incorporation process Compact cortical bone grafts strongly impede the ingrowth of fibrovascular tissue essential for revitalisation of the graft The cortical and cortico-cancellous grafts mainly consolidate to the host bone and show superficial revascularisation with slow and limited incorporation and remodelling, leaving substantial amounts of dead bone graft

that may fail due to fatigue micro-damage [6 17 30] These grafts, therefore demonstrated inferior clinical long-term results [18 21 26] In contrast, the porotic structure of a trabecular graft allows a more rapid revascularisation than the tight cortical bone Animal experiments using morsellized impacted cancellous bone allograft in the acetabulum and femur demonstrated complete graft incorporation and remodelling after 12 weeks [37 38] In human biopsy material, the cancellous morsellized graft had consolidated to the host bone, was revascularized and finally remodelled into a vital trabecular structure [27 32]

A prerequisite for incorporation of the graft is a stabile environment to support the osteogenic tissue Therefore the graft as well as the host bone has to provide for a stable structure The fresh-frozen or freeze-dried bone grafts, either as structural graft or morsellized impacted graft, can provide for a mechanically stable graft The stability of the graft is of utmost importance to bridge the small graft-host interface with vascular and osteogenic elements, resulting in graft-host bone consolidation [42 45] Lack of stability, which is a reasonable risk using large fragment grafts, can lead to resorption of the graft

Several experimental studies have demonstrated the importance of mechanical loading conditions on the process of fracture healing [14 15] and trabecular remodelling [13] In immitiation of these findings, a role of the mechanical environment in the incorporation process of bone grafts, can be expected A clinical observation is that lack of loading, due to instability of structural graft or stress-shieling of the bone graft, lead to resorption of the graft Also in an animal experiment, to test the femoral morsellized bone grafting technique, it was found that prosthetic design factors may affect the graft incorporation process [39] The stress pattern as known from Finite Element Analysis [19], was thought to be responsible for the level dependent graft incorporation

The host bone environment is also important in providing sufficient vascular and cellular elements to revitalize the dead bone graft An often stated opinion is that the loosening process of failed arthroplasties leads to a sclerotic and avascular periimplant bone This situation potentially could jeopardize a rapid and sound graft-host bone consolidation and incorporation Only a few studies described the periprosthetic bone after implant failure regarding trabecular thickening and interface tissue, however, no mention has been made concerning vascularity and osteogenic activity [23 33 35]

The immunological differences between graft and host, i e histocompatibility of the bone graft, is responsible for a cellular and humoral response that could negatively affect bone graft acceptance [7 11 42] The immunological response is directed to cell-surface antigens and depends on the amount of antigen present in the graft Especially bone marrow is rich in antigens Deep freezing and defatting of bone grafts diminish the immunogenic response [31 44] The significance of the immunological activity for clinical failures is yet not clear [20]

The osteoinductive activity of the graft will depend on its capacity to activate the surrounding host tissue for active bone ingrowth The osteoinductive capacity of bone graft is probably directed by growth factors stored in the matrix or generated by growth factors supplied through the new vessels and cellular elements The exact mechanism of osteoinduction is not clear yet, however, factors like Transforming Growth Factor-ß (TGF-ß), Platelet Derived Growth Factor (PDGF), Insuline-like Growth Factor (IGF), Bone Morphogenetic Protein (BMP) and Fibroblastic Growth Factor (FGF) may be involved in this process Various studies have demonstrated positive effects of externally applicated TGF-ß, BMPs and b-FGF on bone graft incorporation and bone healing in animals [1 2 3 24 46] BMPs are found to have osteoinductive effects in human nonunions and maxillofacial bone defect [22 36] Particularly in cases with reduced healing conditions due to unfavourable host conditions, these factors could provide for a more optimal environment for bone graft incorporation

Clinical use of bone grafts in total hip arthroplasty

In the late seventies Harris et al developed a technique to restore deficient acetabuli by using femoral heads in the form of structural corticocancellous grafts, fixed to the pelvis with bolts and screws [16]. This technique became very popular. However, longer term follow-up studies revealed that the structural allograft only could provide for a short term solution [21,26,47]. After 5 years these grafts collapsed, which was to some extent caused by fatigue fracture of the central part of the dead graft, resulting in migration of the acetabular implant.

Coincidental to this 'structural' reconstruction, another operation technique, using 'small-fragment' autologous cancellous bone graft was developed to reconstruct acetabular deficiencies. The first to describe the use of these bone chips were Parker and Hastings [34]. McCollum et al [29] developed a technique using the bone graft fragments like roof-tiles to reconstruct acetabular protrusion. This technique was modified by Slooff and coworkers, using impaction of the morsellized allograft in combination with cemented cups [40]. This biological reconstruction method for acetabular deficiencies, revealed successful long-term clinical results [25,41].

Orthopaedic oncology has had many years of experience with massive 'large fragment' bone grafting in combination with special megaprostheses for the treatment of patients who require femoral reconstruction. Also at the femoral side, incomplete or unpredictable incorporation of the solid bone graft was the biggest problem and the use of massive structural grafts in combination with large cemented prostheses resulted in frequent complications like fractures and graft resorption [6,28]. Supported by the good clinical results after acetabular reconstruction with morsellized bone grafts and cement, a analogous technique was developed for the femoral side [9]. Once again, favourable clinical results were obtained with this femoral impaction technique in combination with cement [4,10,41,39a].

Aim of the study and structure of the thesis

From experimental and clinical observations in the Department of Orthopaedics in Nijmegen it became clear that the use of morsellized bone graft in combination with cement for prosthetic fixation was successful. To further perfect the application of this type of graft, it is necessary to enlarge our basic knowledge. Hence, the factors involved in its incorporation had to be studied in human and animal experiments to answer the following questions:

- Is the periprosthetic bone a risk factor in the incorporation and clinical success of the impacted morsellized bone grafting technique ?
- Is the incorporation process of impacted morsellized bone graft in human histology similar to animal-experimental findings ?
- What can be the role of commonly used growth factors in the incorporation process of bone grafts under controlled non-loaded conditions ?
- Does the mechanical loading situation influence the bone graft incorporation process?

One of the main indications for using bone grafts is the reconstruction of failed total joint replacements with loss of periprosthetic bone, due to the loosening process. In **Chapter 2** the bone resorption process with the role of clinical, biological and mechanical factors is reviewed. The effect of the loosening process on the quality of periprosthetic bone is described in **Chapter 3**. In this chapter the quality of periprosthetic bone present at primary total hip arthroplasty and at revision surgery after aseptic implant loosening is described histologically and morphometrically, particular notice has been made of the vascularity of the host bone. In **Chapter 4** the histology of im-

pacted morsellized bone graft in the revised acetabulum in humans, after variable post-operative time-periods is described. To investigate the role of the biological factors, i.e. the growth factors and the influence of loading on graft incorporation under controlled experimental conditions, two animal models were developed. The animal models and experiments are described in the **Chapters 5, 6 and 7**. The bone chamber model is described in **Chapter 5**. In **Chapter 6** the influence of growth factors like BMP-2, TGF-ß and b-FGF on bone graft incorporation in non-loaded conditions is described. In **Chapter 7** the role of controlled mechanical loading conditions in the incorporation process of morsellized grafts is described.

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CHAPTER

BONE RESORPTION PROCESS AROUND STABLE AND ASEPTIC LOOSENED TOTAL HIP ARTHROPLASTIES. A review.

Nancy M.P. Lamerigts, Pieter Buma, Tom J.J.H. Slooff.

Journal of Material Science: Materials in Medicine (In Press)

INTRODUCTION

Total hip replacement is generally a very successful operation in orthopaedic surgery However, a major long term complication is massive loss of bone stock in well functioning and failed arthroplasties, both in the acetabulum and femur

Loss of bone stock around arthroplasties is thought to be a multifactorial process Several studies have been conducted to elucidate the resorption events which occurr in the loosening process Patient variables, chemical and physical properties of the biomaterials, biomechanical factors and immunological events have all been found to influence the loosening process There still is controversy about the main factors involved when these processes occurr around prosthetic material This has led to an enormous and still increasing number of different types of prosthetic design and biomaterial specific modifications, adjusted to the prevailing opinions of the biomedical industry, research group or surgeon

We reviewed the incidence of resorptive changes around hip arthroplasties An update is given of basic knowledge about the bone resorption process and the current theories about factors involved in bone resorption processes around stable and aseptic loosened arthroplasties New approaches to unanswered questions about osteolytic phenomena in prosthesiology and potential interventions in peri-implant bone resorption are discussed

BONE RESORPTION AROUND ARTHROPLASTIES

Clinical incidence and histological appearance

Charnley [19] was the first to describe periprosthetic bone loss He observed osteolysis around a few cemented femoral stems and stated that infection, even without bacterial confirmation, was the most likely aetiology Harris et al [46] also published an early report on osteolytic phenomena around cemented femoral prostheses, but their findings suggested a foreign-body reaction in relation to loose femoral stems

Radiolucencies around prostheses can be observed on an X-ray as dark lines or discrete areas between the implant and bone Localized bone resorption, cystic erosion and scalloping are expressions that have been used synonymously to describe well-demarcated local lucencies, which are absent on the direct postoperative X rays [2 56 77 79 130] Diffuse linear radiolucencies are considered to represent an expansion of the periprosthetic cortical bone [110 128]

Cemented implants

Bone resorption has been observed around loose [18 106 109 119 145 146] and stable cemented implants [2 64 86] with cystic and diffuse linear radiolucencies in both conditions It has been reported that the linear radiolucent areas were distributed along the cemented prostheses with no favourite localization [109 128] The osteolytic areas were mainly located around the distal stem and on the medial side of the proximal femur, often in cement-deficient areas [2 56 77 79 86 130 152]

In some studies, a correlation was reported between the radiographic and histological features of the bone resorption areas In osteolytic areas at cement deficient sites, granulomatous tissue with wear particles was observed by Anthony et al [2] and Kwong et al [77] Longitudinal bone resorption along a loose cemented prosthesis was mainly filled with fibrous tissue, according to Santavirta et al [126] However, in another study with a comparable patient group, duration of implant and linear bone resorption appearance, macrophages and wear particles were observed [128] Tissues derived from focal osteolytic areas of revision specimens were found to contain macrophages and wear particles [128] Retrieval studies also revealed histiocytes and wear debris in tissues

obtained from areas of bone resorption far from the articular surface around mechanically-stable cemented implants [2 64 86 128] However, other retrieval studies on stable cemented femoral prostheses regarded the linear and cystic radiolucencies to represent osteoporosis of the cortex rather than the presence of fibrous tissue The cement layer had become enveloped by a neocortex which was connected to the endosteum by thin trabeculae [19 62 63 77] Thus, in the evaluation of linear radiolucencies special care must be taken not to define a well fixed prostheses as being loose

In 10-15 year follow-up studies of successful arthroplasties, the incidence of radiolucencies of any type around cemented femoral components was 20 % [66 149 152] and 35-90 % in prerevision arthroplasties [105 109] Focal osteolysis started to develop 3 years after insertion of the cemented arthroplasty [85 130]. According to Huddleston [56] the cystic radiolucencies tended to expand without exception, whereas Maloney et al [85] observed progression at a variable rate The presence of focal endosteal excavations had no influence on the risk of aseptic loosening of rerevision femoral arthroplasties, according to the survival analysis by Retpen and Jensen [115] In primary arthroplasties on the contrary, endosteal cavitations have been identified as a "risk" feature [105] Because the patient groups and analysis methods are not comparable, no conclusion can be drawn from comparing these two studies It has been demonstrated that extensive linear lucencies are a significant detrimental factor in the long-term survival of cemented femoral implants [72 118], especially if the lines are divergent from the implant [15] The contradictory reports on the appearance of femoral osteolysis with its histological aspects and its influence on the survival time of an implant, emphasizes the need for retrieval studies that correlate individual serial clinical radiographs with the corresponding histological findings These studies should investigate whether or not we are dealing with separate entities of radiolucency in with respect to histological appearance and potency of progression

Reports on the appearance and progression of osteolysis in studies on the acetabular side are more uniform Linear radiolucencies of 1 mm or larger around cemented acetabulae are very common without any sign of loosening The incidence of this phenomenon varies from 15 % at 3 years follow-up [44] to 30-60 % after 10 years or longer [66 103 153] It has been suggested that progression of the larger lesions by more than 2 mm is predictive for acetabular loosening in the future [36 44 66] The process of bone resorption starts circumferentially at the intra-articular margin and progresses towards the dome of the implant [44 129] The superior acetabular margin displays the highest incidence of radiolucent lines [36 44 66] The fact that the incidence and size of osteolytic areas increase with time, means that the acetabulum is more vulnerable for failure in the long term

Noncemented implants

Initially, the phenomenon of osteolysis was mainly thought to be caused by the cement layer This conception stimulated the development of cementless osseous integrated implants, which eliminated the use of polymethylmethacrylate as a method of fixation However, bone resorption also developed around these noncemented protheses, even in clinically well-functioning implants [9 29 68 84 122]

Radiographic analysis of clinically well-functioning, proximally porous coated femoral components, demonstrated areas of focal and linear osteolysis in 20 % of the hips The osteolytic process started to develop 2-3 years postoperatively, mainly in the calcar region and around the distal femoral component [47 68 84], Tanzer et al [136] observed more severe osteolysis along loose components, but noticed progression in 90 % of all their total hip arthroplasties, whether stable or loose In the study by Brown and Ring [9],

fully-coated prostheses with a stable distal fixation demonstrated severe osteolytic changes in the calcar region Engh et al [29], on the contrary, observed only slight bone resorption with comparable fully-coated prostheses An important difference was that the implants used by Brown and Ring had a polyethylene femoral head Smooth cementless femoral components demonstrated extensive radiolucencies in 20-40 % of the femoral implants in the calcar and tip region, only after 2-6 years follow-up, which correlated with poor clinical results [26 108] The isoelastic prostheses showed extensive bone resorption in more than 40 % of the patients after a follow-up of at least 5 years [122]

Only a few studies made a correlation between radiolucencies around cementless femoral components and the histological features Studies on stable noncemented implants revealed dense fibrous tissue in non-ossified areas [30] In focal osteolytic areas, histiocytosis and particulate material was observed [84]

Acetabular cystic osteolysis has also been observed in clinically stable noncemented implants; this developed 1-5 years postoperatively Histologically, these lesions contained macrophages, giant cells and particulate debris of polyethylene and metal [11 86] Acetabular osteolysis was often located adjacent to screws and was associated with radiographic evidence of gross wear of the polyethylene liner [11 68 86] In a comparison between the radiological examination results of stable retrieval acetabulae and the histological findings, Engh et al [31] observed dense and well-organized fibrous tissue without granuloma formation or wear particles in the diffuse radiolucent areas Acetabular retrieval studies combined with detailed clinical radiological follow-up are needed, because it is still unclear whether the various types of radiolucency have different clinical implications

In summary, osteolytic areas with varying appearances, variable progression rates and different histological features have been observed It is important to extent our knowledge of the radiographic risk factors, i e osteolytic appearances, which indicate loosening, so that revision surgery can be planned at the right time This will not only prevent unnecessary interventions, but also massive bone loss

Cemented versus noncemented prostheses

Comparison of studies with respect to the incidence, localization and extension of osteolysis that occurs around cemented and noncemented implants can be biased by multiple variables, such as patient characteristics, surgical technique, composition of the implants and follow-up period Therefore, no comparisons have been made between foregoing discussed cemented and noncemented implants

In a retrospective, matched-pair study on the prevalence of femoral osteolysis comparing cemented and noncemented prostheses, osteolysis had only developed in the noncemented group after a follow-up of 4 years [39] In a prospective study by Wixson et al [150], a comparison between the proximal cortical bone resorption around cemented and uncemented prostheses revealed a varying degree of osteolysis, which tended to be more severe around the uncemented femoral stems than around the cemented ones This difference was explained for by the easy access of very small particles into the bone-prosthesis interface, which in turn could stimulate the bone resorption process This process is explained more extensively in the next paragraph

Compared to cemented acetabulae, the noncemented showed a lower incidence of radiolucent lines and resorption areas [68 150 154] However, postmortem studies on well-functioning noncemented acetabulae and the findings at revision surgery indicated that routine X-rays led to an under-estimation of the presence of bone loss and an overestimation of the occurrence of bone apposition [31 86].

Granulomatous osteolytic lesions

Aggressive granulomatous osteolytic lesions are described as a separate item, because they appear to have a different way of presentation compared to the above-mentioned radiolucencies.

Granulomatous osteolysis has a very progressive nature and presents on a standard X-ray as localized ovoid tumour-like aggressive bone resorption of periprosthetic bone. It has been observed around both cemented [64 126 135] and noncemented prostheses. The lesions are often multifocal, with the proximal femur and supra-acetabular region as common sites, both in cemented and noncemented implants [125 126,135]. These lesions are responsible for 5% of revision operations for a loose total hip [86 125 135] and become visible 3-5 years after the primary operation [86 126].

These cyst-like lesions invariably contain granulomatous tissue with sheets of macrophages, foreign-body giant cells and wear particles, irrespective of whether the prostheses were cemented, noncemented, symptomatic or asymptomatic [64 86 126].

The fact that these aggressive lesions have a local presentation and mostly appear after only a few years of implantation, allows the surgeon to follow early postoperative radiolucencies with plain X-rays without any additional investigations or interventions.

BONE CELLS AND THE LOCAL BONE RESORPTION PROCESS

To better understand the bone resorption process along prosthetic components, it is essential to gain insight into the origin of bone cells and the factors involved in their regulation. This paragraph does not attempt to incorporate all aspects of this topic, but is restricted to specific areas that elucidate the orthopaedic 'osteolytic' literature.

The origin of bone cells

Four types of bone cell can be distinguished in compact and trabecular bone, namely the osteoblast, osteocyte, osteoclast and bone lining cell ***(Fig. 1)***.

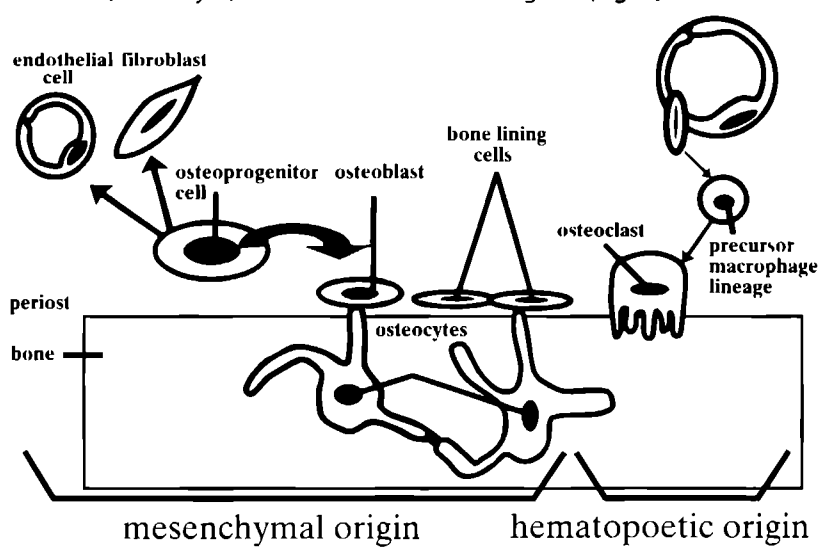

Figure 1
Origin of bone cells
Osteoblasts derive from undifferentiated mesenchymal cells After the osteoblasts are surrounded by mineralized matrix, they are designated osteocytes Osteoclasts differentiate from precursors of haematopoietic origin The origin and fate of bone lining cells is not yet established

Osteoblasts are derived from relatively undifferentiated osteoprogenitor cells of mesenchymal origin These cells can also develop into endothelial cells, fibroblasts or reticulum cells (the supporting cells of the bone marrow), depending on local physical and biochemical influences [104] When osteoblasts are surrounded by mineralized matrix they are designated osteocytes

Bone lining cells are flat, elongated cells that cover most of the temporary non-remodelled endosteal bone surfaces There is still uncertainty about their function and origin, but they may play a role in the functional syncytium of osteocytes because of contact over numerous cellular gap junctions Their role as a progenitor of osteoblasts has not been established [90]

The osteoclast precursors are of haematopoietic origin and are related to the monocyte-macrophage lineage [92] The initial pathways of osteoclast and macrophage differentiation are identical, but the final pathways of differentiation are different [4 61] No direct descendence of osteoclasts from mature macrophages has ever been demonstrated [13]

The biology of the local bone resorption process

Local physiological bone resorption is influenced by various factors, such as the mechanical loading situation, cytokines, growth factors and systemic hormonal stimuli Apart from synthesizing bone matrix, osteoblasts also affects the resorption process by means of paracrine mechanisms, which influence osteoclasts ***(Fig. 2)*** After receiving the signal to initiate the resorption process, osteoblasts show cell retraction, which exposes non-mineralized collagen This thin osteoid layer is then removed by an as yet unidentified mechanism of collagenase action, wich exposes mineralized bone to osteoclasts [16 117 141]

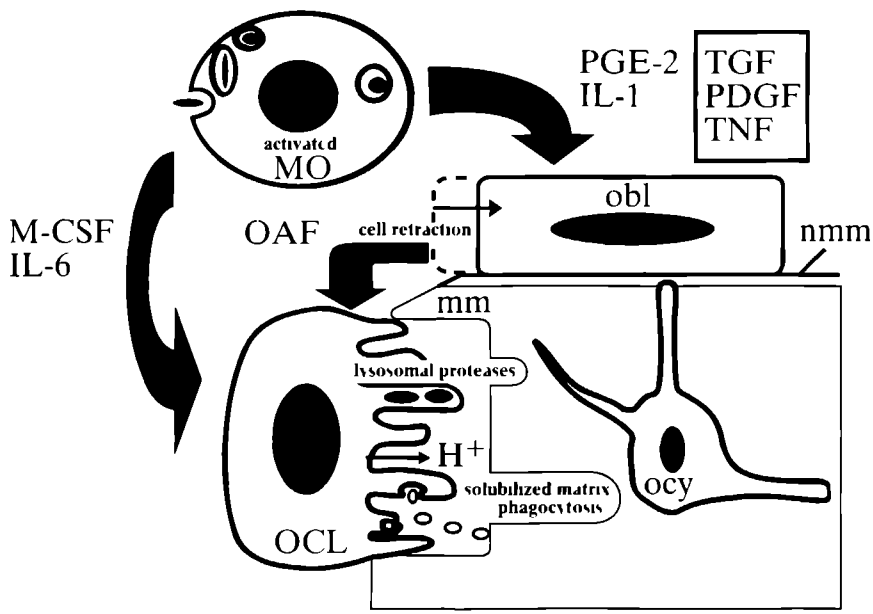

Figure 2

Overview of the local bone resorption process

Osteoclast (OCL) activation directly depends on activation by osteoblasts (obl) Also activated macrophages (MO) enhance the resorption process by stimulating osteoblast activity and osteoclast formation Several cytokines growth factors (M CSF IL 6 PGE 2 IL 1 TGF PDGF TNF) and Osteoclast Activating Factors (OAF s) play a role in the complex interacting system mm= mineralized matrix nmm= non mineralized matrix

Osteoclast Activating Factors (OAFs), excreted by osteoblasts, further stimulate the resting osteoclasts to form active multinucleated bone resorbing cells with a ruffled border As yet these OAFs have not been fully identified, but they are probably metabolites of the arachidonic acid chain [88 89] Although macrophages and osteoclasts have a common precursor, living bone is only resorbed by osteoclasts and not by macrophages [13 106] Dead bone, on the contrary, is readily absorbed by monocytes [13 91]

Cytokines are short-range soluble mediators that are released by cells, which modulate the activity of other cells The term cytokines has been used more generally to include products originally described as monokines, lymphokines and growth and differentiation factors Several cytokines are potent mediators of bone resorption, such as Interleukine-1 (IL-1) α and ß, Tumour Necrosis Factor (TNF) α and ß and Interleukine 6 (IL-6) Transforming Growth Factor (TGF) α and ß and Platelet Derived Growth Factor (PDGF) have also been observed to play a modulating role in the resorption process [116]

In-vitro studies have demonstrated that macrophage excretions, such as Prostaglandine E 2 (PGE2) and IL-1 activate the resorption process by affecting the osteoblast Part of the IL-1 effect on bone is prostaglandine-mediated [94] Macrophages also produce Macrophage Colony Stimulating Factor (M CSF) [73] and IL 6 [76 120] that enhance osteoclast formation

PGs are important local regulators, because every stimulator of bone resorption has been shown to increase prostaglandine production PGs can act as a general amplification system for resorption stimuli [70] Local PG concentrations can be raised by hormonal stimuli [70 111] and by local factors including IL 1 [127 140], TNF [138], TGF- α [139], PDGF [137] and mechanical force [28 97] It has not yet been established which bone cells are responsible for PG production [94], but osteoblasts in culture seem to be a likely source of PGs [32 100]

PGs can have dual effects in the bone resorption process *In-vitro* studies have shown that, besides its resorptive activities, PGE2 also has a stimulating influence on bone growth, depending on its concentration [21 112] This phenomenon has also been observed *in-vivo* with the factor TGF-ß [87 99] Interactions between cytokines are important in modulating bone resorption Il-1 and TNF can influence the PGE2 concentration Il-1 can interact synergistically with TNF and TGF-α to increase bone resorption [81 133]

Local bone resorption with cytokines and growth factors is a complex interaction system The relationships between the factors involved in this complex biological system have not yet been fully clarified

Mechanical influences and bone resorption

Mechanical loading plays an important role in the differentiation, growth and remodelling of bone Numerous mathematical and experimental attempts have been made to describe the relationship between mechanical loading and bone resorption

Kufahl and Saha [74] developed a mathematical model to describe the stress-induced flow They stated that a lack of stress-generated intracellular fluid flow, because of nonloaded conditions or interruption of inter osteocytic contact, can lead to bone resorption Frost [34 35] put forward a mathematical theory about the 'Mechanostat' according to Wolff's law The response of bone to mechanical strain is considered an intrinsic property of the bone He attributed the adaptations of bone architecture and mass to its typical mechanical environment, based on sensor and actor presence in 'mechanical' units Recently, a model which describes the excitation of osteocytes by bone fluid shear stresses has been developed [144]

Support for these theories can be found in the results of *in vitro* experiments Several investigations have demonstrated inhibition of the osteoclastic resorption process under intermittent strain induction [12 71 98] *In-vitro* cyclic stretching of osteoblasts

demonstrated a significant increase in mitotic activity, but not in alkaline phosphatase activity [98] Osteoblasts have also been found to respond to cyclic mechanical stretching *in-vitro*, with orientation of the cells perpendicular to the strain field applied [12] After mechanical stimulation several non-defined bone resorbing factors are released [124] *In-vitro* studies have also confirmed the production of PGs by bone cells after subjection to loading or stretching [8 28 112 113]

Very little is known about cellular interactions during mechanical excitation Osteocytes, bone lining cells and osteoblasts may communicate via paracrine effects of produced signalling factors Direct communication may occur via electrical currents and cytoplasmic substances, that are transferred through the syncytium via gap junctions [8]

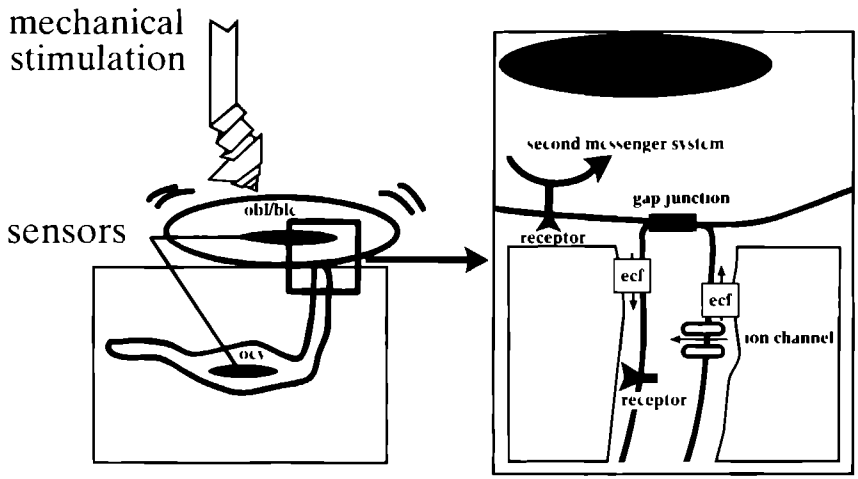

Figure 3
Hypothetical mechanism for mechanical stimulation of bone cells Stretch-sensitive ion-channels or receptors sensitive to force variation can lead to second messenger cascade activation resulting in remodelling activity obl= osteoblast, blc= bone lining cell ocy= osteocyt ecf= extracellular fluid stream

Although it is still unclear how mechanical stress is transducted into cellular biochemical signals, some models describe hypothetical mechanisms ***(Fig. 3)*** Stretch sensitive ion channels, which have been observed in the cell membrane of osteoblasts could be responsible for the activation of osteoblasts [24] Another model of transmembrane stimulation was described by Sandy et al [123] By means of *in-vitro* experiments they demonstrated that specific receptors, which are sensible to force variation, activated intracellular messenger cascades in the osteoblast

In the foregoing, various factors, such as the monocyte-macrophage system, cytokines, prostaglandines and mechanical loading, have been discussed extensively, to elucidate more clearly in the next paragraph their role in the bone resorptive phenomena along total hip arthroplasties

FACTORS INVOLVED IN THE BONE RESORPTION PROCESS IN TOTAL HIP ARTHROPLASTIES

Wear particle characteristics and bone resorption

Wear is a process of particle generation from surgical implant materials, owing to contact abrasion between two materials, or material failure because of fatigue, fretting or corrosion [147 148]

Wear particles around total joint arthroplasties mostly consist of metal, high density polyethylene (HDPE), polymethylmethacrylate (PMMA) and hydroxyapatite (HA) granules. The particles can be generated between bearing surfaces or between debonded noncemented prostheses and bone ***(Fig. 4)***. At other interfaces, i.e. bone-cement and implant-cement, particles may also be generated because of slight movements between the surfaces. The new generation of modular implants and the use of fixation screws are also a source of wear particles [7]. Entrapment of cement or coating particles between the articulating components of the arthroplasty can give rise to so called third-body wear [5].

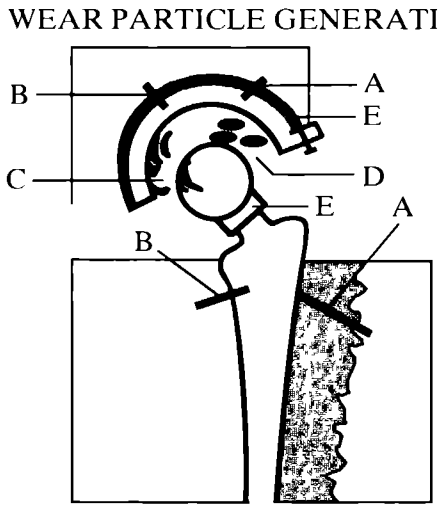

Figure 4
Sources of wear particle generation
A = cemented interface, bone-cement-metal stem/polyethylene cup
B = cementless interface, bone-metal stem/metal backing/porous coating
C = articular surface roughness
D = thirth body wear
E = modular system/screw fixation

The release of biomaterials in particulate form is responsible for the increased invasion of phagocytotic inflammatory cells and granuloma formation. The periprosthetic particles are phagocytosed by macrophages and giant cells, which leads to the release of soluble inflammatory mediators, such as PGE2, IL-1, IL-6 and TNF [65,96]. Cytokines are known to stimulate granuloma formation [75], and as was described extensively in the former paragraph, they also activate bone cell participation in the bone resorption process.

Animal experiments have stressed the importance of particle presence in the generation of osteolytic areas along implants. In the absence of mechanical loading, a thin fibrotic membrane was observed around implanted bulk material with an intact bone mass, whereas a florid foreign body giant cell reaction with osteolysis of the surrounding bone was demonstrated after implantation of the same amount of material, but in a particlate form [42,43,55].

Only particles of a certain size stimulate macrophages to phagocytosis and the secretion of cytokines [51,53,54]. In an *in-vitro* study, Glant et al.[38] observed a maximal bone resorption response to titanium particles of 1-3 μm with a concentration of 10-15 particles per cell. The surface area of the particles also seems to be important in the ultimate inflammatory response of macrophages. Irregularly shaped PMMA particles elicited a significantly higher cytokine production than spherical particles [37]. Also for bulk material evidence was found that activation of macrophages varies with physical properties such as surface energy and roughness rather than chemical nature of the biomaterial [95].

The maximum concentration of particles, that stimulate macrophages, is different for the various biomaterials [96]. The *in-vitro* study by Glant et al.[38] demonstrated that

the bone resorptive activity of titanium particles was significantly higher than that of polymer particles Other authors believe that a combination of materials with a pattern of wear and specific number and size of the wear particles have a more adverse affect on the *in vivo* reaction than particulate debris from a specific material [52 54]

Wear debris induced osteolysis in clinical studies

The results of clinical studies have provided support for the theory that induction of bone resorption depends on the quantity of particles [49 128] Osteolysis in noncemented protheses was found to be directly correlated with the amount of polyethylene wear of the acetabular cup, this was also verified histologically [67] When the polyethylene wear of a cemented acetabulum exceeded 2 mm, proximal femoral lysis phenomena were present without exception [80 142] Charnley did not find a relationship between the amount of socket wear and amount of absorption of the calcar femorale [19]

Several authors have studied the osteolytic potential of tissue obtained from bone resorption areas from cemented and noncemented prostheses, by means of cytokine essays and *in-vitro* bone organ culture The interfaces of failed cemented prostheses demonstrated IL-1, PDGF [65], TNF and PGE2 production [3] The membranes around osteolytic areas of failed noncemented prostheses contained large quantities of IL-1 [67] A comparative study on noncemented prostheses with or without focal endosteal bone loss, demonstrated more macrophages and small particles and greater Il-1, Il-6 and TNF activity in the group with focal osteolysis [20] Other studies did not find a higher cytokine concentration in particle-burdened tissue [23 67] In the studies by Ohlin [101 102], the mediators released from the joint capsule demonstrated greater bone resorptive activity compa red to the bone cement interface membranes It is possible that the larger particle burden in the capsule was responsible for the greater abundance of activated macrophages and subsequently, higher cytokine production

Several hypotheses have been put forward in clinical studies to explain the variance in the localization of osteolysis Wear particles can be released locally or transported along the bone-implant or cement prosthesis interface by a pumping mechanism [2 33 50 52 79 86 128] This theory was supported by the findings in a recent study by Goetz et al [39], in which the lower incidence of osteolysis in cemented femoral prostheses was explained for by the lack of wear particle access to the periprosthetic interface, because of the tight bone-implant fixation with a third-generation cementing technique In the same context, several authors observed extensive osteolysis around smooth cementless femoral components with a poor bone-implant fit [26 108 122]

The interindividual differences in the quantity and localization of osteolysis in patients, could also be the consequence of non-phasic fluid flow through the so-called effective joint space Variation in the intracapsular pressure and obstruction of the flow by local implant-bone connection, may be responsible for different patterns of particle transport along the bone-implant interface [128]

Other authors have hypothesized that wear particle transport along the perivascular lymfatic vessels reaches an equilibrium Ones the transport capacity has been exceeded, the particles would remain locally and induce a granulomatous reaction with subsequent bone lysis [84 145 148] Recently the histopathology of sinus histocytosis has been described Metal particles presence has been described in the pelvic lymph nodes and lymphoreticular tissue after metal total hip replacement [1 78]

Herman et al [48] introduced another theory to explain the various osteolysis localizations the fluid shift theory Pressure changes in the joint and periprosthetic fluid would facilitate the wide-spread distribution of bone resorbing factors

Mechanical factors involved in the bone resorption process around prosthetic material
Bone cells are sensitive to mechanical load with subsequent biological responses This is also the case with the bone cells in the changed biomechanical environment around prosthetic material So far we know very little about the local *in-vivo* respons of bone cells in changing loading conditions and it is not possible to predict the bone resorption result from a combined biomechanical biological point of view

On the basis of Wollf's law [151], which stated that the structure of bone adapts in accordance with an altered mechanical environment, computer models have been developed, using the Finite Element Method, to predict the long-term behaviour of periprosthetic bone along various types of prothesis [60] In an animal experiment, Finite Element simulations of adaptive bone remodelling processes around noncemented implants showed close similarity with the cross-sectioned animal material [143] In an attempt to validate the prediction of bone loss, human periprosthetic bone of noncemented retrievals described by Engh et al [31], was compared to biomechanical simulation models Although certain assumptions were made, the same trends in bone loss were observed [58]

A decrease in loading will lead to bone resorption, which is what happens when stress is distributed along the stem to the distal femur This so-called stress shielding is a process of strain-adaptive bone resorption, in reaction to abnormal stress distribution and leads to bone loss in the proximal femur The main prosthetic factors responsible are stem stifness [6 134] and bonding characteristics of the implant Human retrievals with a stable cemented prosthesis in situ, which were subjected to strain gauge studies, revealed that there was marked stress shielding in de proximal medial femoral cortex, even long after implantation [83] The cement layer had become enveloped by a neocortex which in turn was connected to the cortex by trabecular struts There is no evidence that stress shielding limits the longevity of cemented prostheses [45] However, more substantial and extensive bone loss is observed around noncemented implants, because they are more rigid, distributing the stress along the stem to the distal femur [29 57 121]

In acetabular reconstructions there is a concentration of stress along the superior edge of the acetabular wall Interface radiolucency usually starts at the rim of the acetabulum and progresses towards the dome [129] This observation supports the view that resorption and loosening are the consequence of mechanical overload and instability at the rim as well as stress shielding of the subchondral bone in the remainder of the acetabulum [22]

According to some authors, not only bone loss because of stress shielding, but also localized osteolytic areas around a prosthesis can be explained by changed loading conditions Micromovements between the cement-implant or implant-bone interface and shear stresses, are the main initiating factors in the osteolytic process Perren [107] imitated this micromovement-induced bone resorption in an animal experiment Huiskes and Nunamaker [59] correlated the quantitative interface stress patterns with the histological results in an animal experiment Mechanical overloading, so-called peak stress, was correlated with resorption phenomena, which depended directly on the design of the prosthesis The increase in lucent lines along a lateralized femoral component may reflect an increase in shear stress at the bone-cement interface, the latter being generated by an increase in the bending forces of the longer lever arm of the prosthesis [79] According to Carlsson et al [15] and Huddleston [56], micromovements alone could explain the high incidence of cystic lesions around the distal end of the femoral component if there is insufficient cement Maximum loading of the metal-bone interface would activate local bone resorption However, isolated cysts around the proximal two thirds of the femoral component and the low incidence of lysis around the proximal edge of the femoral compo-

nent with loads equal to those at the tip, cannot be explained by this mechanical theory

The former theories are based on direct mechanical influence of bone cells However, from a biological point of view focal osteolysis can occur because of mechanical loading, but with an indirect influence on bone cells Local macrophages that are attracted to the environment around biomaterials with tissue necrosis, a low oxygen concentration and a low pH [131], can be activated by motion, to produce cytokines in the absence of particles [132] Motion between the implant and the surrounding bone and fibrous tissue can also lead to the formation of a pseudosynovial membrane, which consists of cells that resemble synovial lining cells and are capable of producing bone resorptive cytokines in the absence of wear particles [27 40 41].

From the enormous amount of literature on this subject, it is not possible to discern what relative contributions micromovements and particulates make to incite the osteolytic response This can be explained by the fact that when micromotions occur *in vivo*, conditions are present inherently to promote the production of wear particles Greater knowledge of how mechanical factors influence the bone resorption process will lead to a better understanding of the influences of implants on peri-implant bone cells and macrophages

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE EXPERIMENTS

Osteolysis is a major complication in total hip arthroplasties In this review various factors involved in the generation and regulation of the bone resorption process were highlighted A great deal of attention was paid to basic knowledge about the resorption process, the cells involved and cellular factors, because these are the major keys in understanding the detrimental process in periprosthetic bone

Combining the different views about the osteolytic processes along prosthetic implants led to the generally accepted view that bone resorption along implants is a

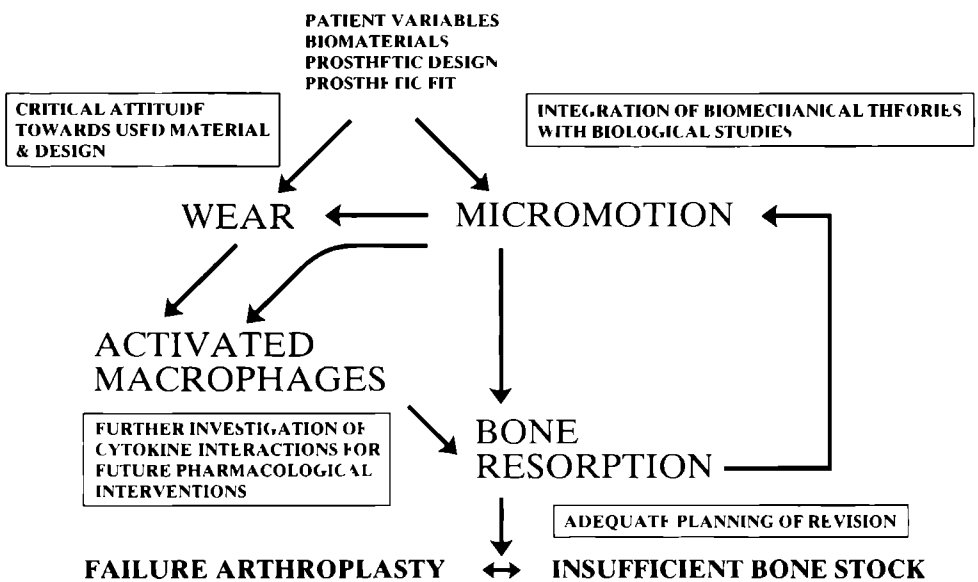

Figure 5
Scheme of the multifactorial periprosthetic bone resorption process with fields of attention in rectangular boxes concerning clinical intervention and further clinical and experimental investigations

multifactoral process in which all the recognized biological and mechanical factors play a role in an early or later phase and finally act synergisticly in aggravating bone loss ***(Fig. 5)***.

Clinical studies were described in which osteolysis was observed along different cemented as well as noncemented prostheses. No difference was observed in the appearance interval and types of osteolytic lesion between the two fixation principles. Generally the local osteolytic lesions contained macrophages with wear particles, whereas linear lucencies composed of fibrous tissue without wear debris presence. In clinical follow-up studies which focuss on the osteolytic process it is important that reports provide definitions of radiographic entities so that clinical and histological results of similar publications can be compared.

Extensive analysis of the sequence of osteolytic events in retrieved material with information of relevant biomechanical and biological influences, will throw more light on the *in-vivo* behaviour of the biomaterial and periprosthetic bone mantle. The observation of cortical osteoporosis, which presence and relevance was explained for by different view points, requires integration of fields of attention.

Biomechanical theories should be integrated with biological studies, in order to validate the mathematical predictions.

Improvements can be made to the *in-vivo* mechanical and chemical stability and the reduction of wear particle production via factors such as the consituents, fabrication and prosthetic design. In addition, care must be taken when chosing modular systems for long-term arthroplasties.

Current knowledge about the cytokines, their reaction sequences and dual effect should be further extended. An important field of investigation comprises interactions between the different cytokines that mediate bone resorption processes around prostheses. We could then explore the potential of pharmacological interventions to directly or indirectly affect active cytokines, for example by the selective inhibition of bone-resorption mediators and retardation of the loosening process after the development of osteolytic areas. Indomethacine [37 69], selective osteoclast inhibitors [116] and proton pump inhibition, which have all been fould to prevent osteoclasts from generating an acid environment for bone resorption, are all non-surgical treatments that may delay the detrimental process and therefore deserve more attention in the future.

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CHAPTER

THE VIABILITY OF THE ACETABULAR BONE BED AT REVISION TOTAL HIP ARTHROPLASTY.

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Journal of Arthroplasty (accepted for publication)

ABSTRACT

Loosening of Total Hip Replacements (THR) is often associated with severe loss of periprosthetic bone. The notion exists that the remaining bone is sclerotic, avascular and diplays little osteogenic activity, and that it therefore potentially compromises the revitalization of bone grafts used to restore bony defects. To verify this opinion we studied the bone characteristics in acetabular bone biopsies taken at Primary Total Hip Arthroplasty (PTH) and Revision Total Hip Arthroplasty (RTH).

In 6 PTH patients and in 10 RTH patients acetabular bone biopsies were taken from the roof, the centre and the lower rim of each acetabulum. Specimens were evaluated by light microscopy and histomorphometrically measured for specimen size, bone area, perimeter, active osteoid perimeter, number of vessels and osteoclasts.

The vascularity and vitality appeared to be comparable in the RTH and PTH bone biopsies. However, the trabecular organization of the RTH bone differed from that of the PTH biopsies. In the PTH biopsies, the trabeculae were running perpendicular to the subchondral bone layer, whereas in the RTH biopsies the layers of bone were oriented parallel to the implant surface. There was abundant remodelling activity in the RTH bone, with large quantities of active osteoid and osteoclasts. These histological parameters differed, but not statistically significantly, from the PTH biopsies.

In conclusion, we found that at revision, the acetabular bone was viable with sufficient vascularity and remodelling activity to provide an acceptable recipient host bone bed for revision surgery combined with bone grafting.

INTRODUCTION

Revision total hip arthroplasty is being performed with increasing frequency, mainly because of aseptic loosening of one or both of the components. The loosening process is often associated with severe loss of periprosthetic bone and requires reconstruction of the joint with bone grafts.

A variety of techniques, using different types of bone graft, have been advocated to restore the deficient acetabular bone stock at primary and revision surgery [1,2,3,4]. Irrespective of the type of graft used, long-term clinical success of reconstruction will depend on sufficient vascularity of the host bone bed, which is a prerequisite for successfully consolidation and incorporation of the bone graft. Unfortunately, not all types of bone graft posess similar incorporative and remodelling characteristics, so that finally the clinical outcome will not only depend on the quality of the periprosthetic bone bed, but also on the graft properties.

However, it has often been stated that failure of primary arthroplasty leads to sclerotic and avascular recipient host bone, displaying little osteogenic activity. This situation potentially jeopardizes bone graft incorporation and in time it may also undermine the mechanical integrity of the revision reconstruction. Although a few studies have been performed concerning the description of periprosthetic bone [6,7,8,9], no specific attention has been paid to quantitate the osteogenic activity and vascularity. In order to determine the vitality of periprosthetic bone, we compared biopsies taken from the acetabular host bone at primary arthroplasty to those obtained at revision surgery, using light microscopy and histomorphometry.

MATERIALS AND METHODS

Patients

Biopsies were obtained from six patients who underwent Primary Total Hip Replacement (PTH) and from 10 who underwent revision of an aseptic loosened acetabular hip component (RTH). Patient characteristics are presented in Table 1.

TABLE 1

	Primary THR	Revision THR
Number (N)	6	10
Age (yrs)	52 (36-78)	61 (47-72)
Sex	F (N=6)	F (N=10)
In-situ (mths)	-	102 (9-276)
Primary diagnosis	pCOA (N=2) CHD (N=4)	pCOA (N=5) epiphysiolysis (N=2) protrusio (N=2) RA (N=1)

Notes F = Female, pCOA = primary Cox Osteo-Arthritis, CHD = Congenital Hip Dysplasia, RA = Rheumatoid Arthritis

Table 1. *Patient characteristics*

Preparation of bone specimens

Biopsies were taken peroperatively from the acetabulum during PTH before reaming, and from the acetabulum during RTH after the cemented component and soft tissue interface had been removed. The samples were taken from three standardized sites (consecutively operated on by the senior author J.G.) in the superior (I), centre (II) and lower region of the acetabulum (III) ***(Fig. 1)***. High quality biopsies were assured by using a water-cooled diamond-tipped drill (Scientific Developments GMBH, Munich), which avoided trabecular fracturing and core impression during the biopsy procedure. The cylindrical subchondral-trabecular bone biopsies were 6 mm in diameter and a maximum of 6 mm thick. The specimens were immediately fixed in 4% buffered formaldehyde, dehydrated in ethanol and embedded (non-decalcified) in PMMA. Three groups of four consecutive sections (7μ) per biopsy were stained with Hematoxylin Eosin, Goldner-Masson, Tartrate-resistant Acid Phosphatase [5] and Weigert-elastine respectively. The sets of sections were obtained at 100μm intervals.

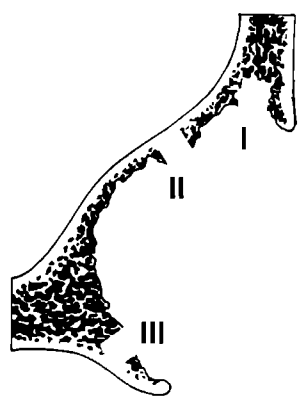

Figure 1
Biopsy sites
I = acetabular roof, II = acetabular centre, III = acetabular floor

Histomorphometric evaluation of bone sections

The following variables were measured in the two groups of patients: *Specimen size=tissue area* (T.Ar), the area of the entire biopsy specimen (mm^2), *Bone Area* (B.Ar), the bone area (mineralized and nonmineralized) in the specimen (mm^2), *Bone Volume* (BV), Bone Area/Tissue Area x 100%, *Perimeter* (Pm), bone borders (mm), *Active Osteoid Perimeter* (O.Pm), osteoid perimeter with cuboid cell seems (osteoblasts) O.Pm/Pm x 100%, *Number of Blood vessels* (N.Ve), number of small arteries/Tissue Area, *Number of Osteoclasts*(N.Oc), number of osteoclasts/Pm.

Histomorphometry was performed using a microscope connected to a computerized video digital tablet system (Videoplan, Kontron Bildanalyse GMBH, Germany). Magnification was 12.5x for T.Ar, 25x for Pm and B.Ar and 100x for N.Ve, O.Pm and N.Oc. The repeated measurement coefficient of variation was 2% for T.Ar, 3% for B.Ar, 3% for Pm, 6% for O.Pm, 18% for N.Ve and 10% for N.Oc.

Statistical analysis

For statistical evaluation the Wilcoxon ranked sum test was used.

TABLE 2

	PTH	RTH
Number of Vessels (#/mm²)		
I	**1.03**	**1.10**
	0.63	*1.46*
II	**1.13**	**1.00**
	1.01	*0.60*
III	**1.22**	**1.27**
	0.72	*1.50*
Bone Volume (%)		
I	**51.7**	**57.6**
	16.9	*19.9*
II	**34.0**	**54.4**
	16.5	*24.5*
III	**39.8**	**51.4**
	22.2	*22.4*
Active Osteoid Surface (%)		
I	**3.2**	**3.8**
	2.8	*5.1*
II	**1.9**	**7.2**
	1.9	*8.0*
III	**1.3**	**3.0**
	1.5	*2.3*
Number of Osteoclasts (#/mm)		
I	**0.14**	**0.24**
	0.14	*0.25*
II	**0.26**	**0.39**
	0 23	*0.30*
III	**0.10**	**0.27**
	0.10	*0.32*

Table 2
Histomorphometric results of primary (PTH) and revision total hip (RTH) group ***Mean*** *and SD*

RESULTS

In the PTH biopsies, the vital bone trabeculae were running perpendicular to the subchondral bone layer ***(Fig. 2A)***. Vital bone was also observed in the RTH biopsies but no normal trabecular organization was present; the layers of bone were oriented more parallel to the implant surface ***(Fig. 2B)***.

The morphological vitality of the bone in the biopsies from the two groups was characterized by the same tend in vascularity at all three sites. No major differences in the number of elastine-staining vessels could be detected between the two groups (P=0.3, P=0.9, P=0.3 for regions I, II and III, respectively; Table 2, **Fig. 2D**).

The highest bone volume was found in the biopsies taken from the superior, load-bearing area I in both the PTH and RTH acetabulae. Although the RTH biopsies appeared to have a more dense bone structure than the PTH biopsies at all three sites, the differences were not statistically significant (P=0.8, P=0.07, P=0.7 for regions I, II and III, respectively; Table 2).

Remodelling activity was most abundant in the RTH biopsies, but not at any predominant location ***(Fig. 2E)***. Morphometrically, the size of the active osteoid surface was the largest in the RTH biopsies taken from the centre (II) and lower areas (III). In the

PTH biopsies the subchondral bone demonstrated the most active remodelling ***(Fig. 2F)***. However, none of the differences were statistically significant (P=0.7, P=0.2, P=0.1, for regions I, II and III, respectively).

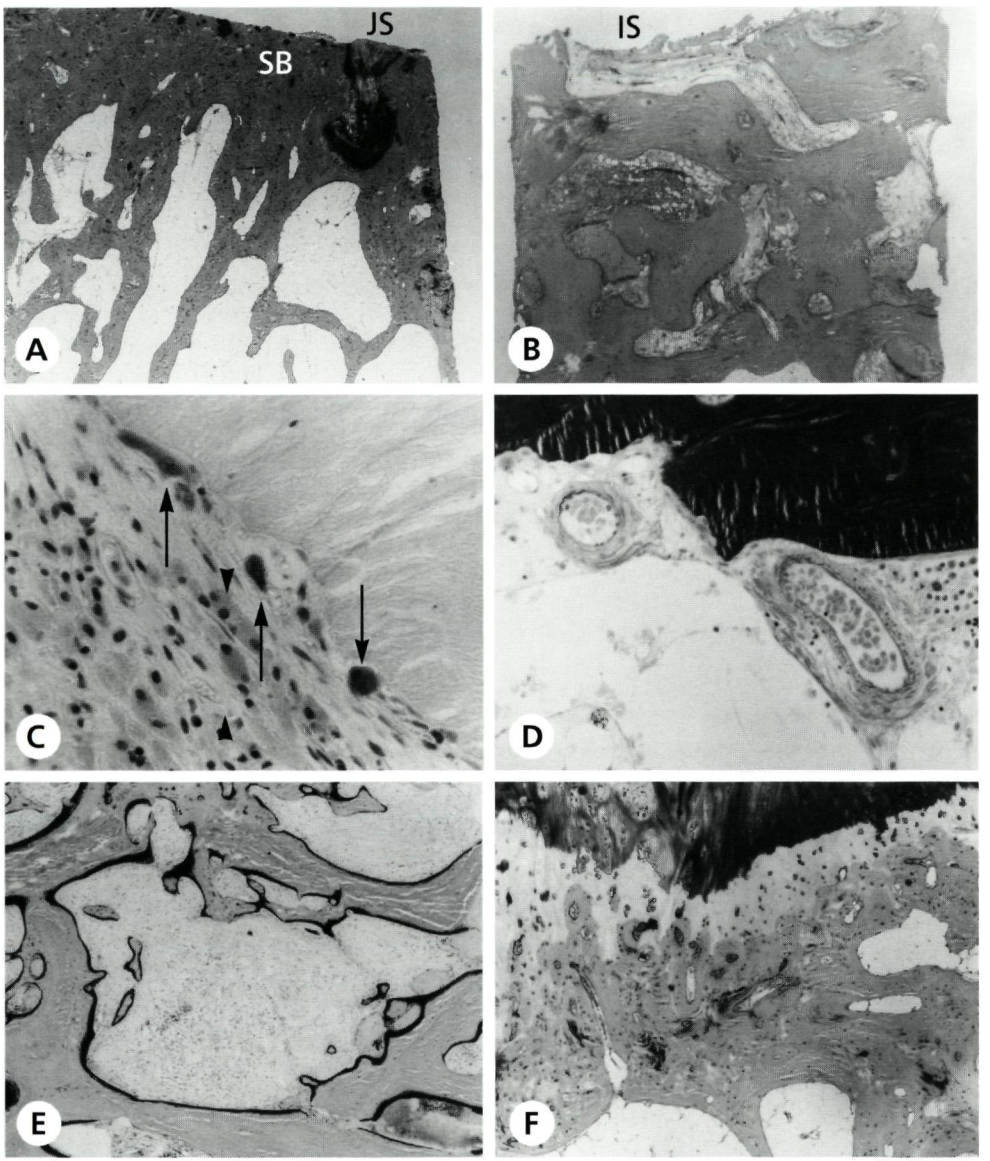

Figure 2
A) *PTH biopsy: trabeculae running perpendicular to the joint surface, sb = subchondral bone, HE 13x;* ***B)*** *RTH biopsy: thickened trabeculae orientated parallel to the implant surface (IS), HE 13x;* ***C)*** *RTH biopsy: TRAP-positive osteoclasts (arrow) on the trabecular surface and faint staining macrophages (arrow-head) with wear particles in marrow spaces HE 100x;* ***D)*** *RTH biopsy: Weigert-elastine positive bloodvessels, Weigert 160x;* ***E)*** *RTH biopsy: extensive osteoid surface, active remodelling, Goldner 40x;* ***F)*** *PTH biopsy: remodelling activity with osteoid staining in subchondral bone layer, Goldner 40x.*

Large marrow spaces, occasionally filled with red or fat marrow, were observed in the PTH biopsies The marrow spaces in the RTH biopsies were mostly filled with fibrous tissue, but they also occasionally contained fat or red marrow, with wear-particle loaded macrophages

A greater number of osteoclasts were observed in the biopsies from all three sites in the revision group, but also these differences were not statistically significant (P=0 5, P=0 5, P=0 3 for regions I, II and III, respectively, Table 2)

Osteoclasts stained vividly red with the TRAP method and were observed on the bone surface (***Fig. 2C, arrow)*** and in the fibrous tissue between the bone trabeculae in both groups The macrophages present in the RTH biopsies only stained faintly orange and could easily be distinguished from the osteoclasts ***(Fig. 2C, arrow head)***

DISCUSSION

Only a few studies have been performed on the vitality of the periprosthetic bone bed In the study by Onsten et al [6] the histological features of the acetabular periprosthetic bone of patients found to have osteoarthrosis and rheumatoid arthritis, were correlated with early implant micromotion, but the authors did not find any differences in movement between the various groups In a clinical study, that considered periacetabular bone quality as a possible factor in cup loosening, the failure rate of revision arthroplasties with bone grafting was no higher than that after primary arthroplasty with bone grating to correct the deficient acetabulum [7] However, the bone quality was not evaluated histologically Another study showed that an insufficient amount of acetabular bone stock combined with inadequate preparation of the acetabulum at the time of surgery had a significant detrimental influence on the outcome of revision arthroplasty [8]

The acetabulum is subject to complex mechanical loading The loading is different in the various parts of the cortical and cancellous bone throughout the acetabulum [10] The high bone density in the biopsies taken from the central part of the revision acetabulae may reflect changes in the loading pattern after cup arthroplasty Evidence of altered loading conditions was also visible in the morphological appearance of the revision bone bed Adaptation to increased stress on the trabeculae, because of a lack of stress-absorbing subchondral bone layer in the bony acetabulum and the occurrence of shear stresses, can probably explain the thickening and the parallel orientation of the RTH trabeculae A similar arrangement of trabeculae parallel to the joint border was also been found in a recent retrieval study by Bos et al [11] Mechanical performance of bone depends not only on the mass of bone present, but perhaps just as importantly, also on its ability to adapt to the specific loads placed on it This should be taken into consideration when histological and/or radiographic characteristics of periacetabular bone are combined with clinical findings [12]

Aseptic loosening of an implant is invariably accompanied by the development of an interface membrane between the moving cup and the host bone The interface tissue consists of fibroblasts and macrophages with variable amounts of wear particles [21] Activated phagocytosing macrophages stimulate the osteoclasts to resorb bone, which leads to a condition of progressive bone loss [22] Abundant remodelling activity and many osteoclasts in the presence of wear-particle loaded macrophages were observed in the biopsies taken from our revision group Micromotions of the cup generate wear particles and cause damage to the trabecular tissue, which is subsequently repaired Abundant remodelling is a manifestation of this loosening process Osteoclasts are attracted and activated by macrophage derived cytokines In order to interrupt this 'resorptive state' it is important to remove thoroughly the interface tissue during revision surgery

The relatively high standard deviations in our measurements are a reflection of the variability in bone structure over small distances. Regionally-dependent structure adaptations are especially found in osteoarthritic bone and in periprosthetic bone. Even in iliac bone, which is not under the influence of variable mechanical conditions, the intersample variation of adjacent biopsies is about 20% [13,14]. However, Ashton-Key et al.[15] found a variability that could run to 1-135% in normal subjects. Although no statistically significant differences could be calculated because of the high standard deviations, we demonstrated a clinically relevant trend of sufficient vascularity in periprosthetic bone.

The successful incorporation of bone graft depends on several factors, such as good vascular and cellular supply from the host bone bed, close graft-host contact, good graft containment and stability of the graft-host bone fixation. Compared to other distinct types of grafting technique used for joint reconstructive surgery, massive structural grafts offer only a short-term solution, and the number of failures tends to rise progressively after about 5 years [16,17,18]. Histologically, osteoconduction only plays a marginal role in the incorporation of massive grafts, with a small rim of remodelling beyond the graft-host interface. Retrieval studies have revealed poor or absent revascularization of the core of the graft after 4 years [19,20]. In our series of patients, more than adequate vascular supply will not form a restraining factor in the incorporation process of the massive structural grafts.

In conclusion, our study demonstrated that the acetabular bone bed at revision surgery, with its specific morphological appearance, was viable, with sufficient vascular supply and remodelling activity to form an acceptable basis for bone grafting combined with revision arthroplasty. The final clinical outcome of joint reconstruction with bone grafting probably depends to a large extent on the level of surgical expertise, including the complete removal of the soft tissue interface and cement, and the type of graft material used.

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CHAPTER

IMPACTED GRAFT INCORPORATION AFTER CEMENTED ACETABULAR REVISION. Histological evaluation in 8 patients.

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Acta Orthopedica Scandinavica 67:536-540, 1996.

SUMMARY

We took core biopsies from the acetabulum in 8 patients after revision with impacted cancellous allograft chips in combination with cement.

Except for one biopsy specimen the graft showed different stages of incorporation. In the specimens taken at 4 months, revascularization of the graft was found. Osteoclasts had removed parts of the graft, while woven bone had formed on the remnants of the graft and within the stroma that was invading the graft. Subsequent specimens showed that this mixture of graft and new bone was in due time remodelled into a normal trabecular bony structure with viable bone marrow that contained little or no remnants of the original graft.

The graft-cement interface was present in 4 biopsies taken at 1, 22, 28 and 72 months. The specimen obtained 28 months after revision showed vital bone locally in direct contact with the cement layer; however, a soft tissue interface predominated.

INTRODUCTION

Whenever possible we apply impaction of morsellized allograft to reconstruct bony defects in the acetabulum [7]. The clinical results of acetabular reconstruction with this technique are very favourable [8,9]. However, unresolved questions include: How fast does clinical incorporation occur? How complete is it. Does it take place in every case with this grafting technique?

We present the histological data of 9 biopsies taken from 8 grafted acetabula, 1 to 72 months after revision.

PATIENTS AND METHODS

Details of the patients' history are given in Table 1.

All patients were revised with the technique described by Slooff et al.[7,8,9]. The acetabular defects were classified according to D'Antonio et al.[2] ***(Table I)***. Most defects were cavitary ***(Fig. 1A; case 1, 3, 4, 6, 7, 8)***. In 2 patients ***(Fig. 1B; case 2, 5)*** combined segmental-cavitary defects (non-contained defects) were reconstructed with Vitallium mesh. The femoral heads received from the local bone bank were morsellized with a rongeur during surgery into chips of about 1/2 cm^3. The quantity of graft used varied from 1 to 3 femoral heads per patient ***(Table I)***. The graft surface was finally covered with a second metal mesh. A polyethylene cup was cemented directly into the graft. The graft thickness was at least 5 mm ***(Figure 1)***.

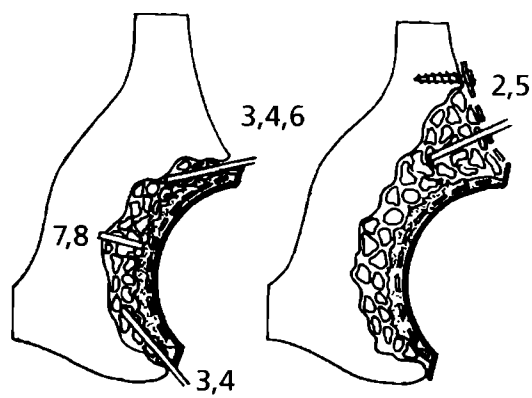

Figure 1
Diagram of locations of core biopsies
The numbers indicate the case numbers.

Re-revision of the grafted acetabula was necessary for the following reasons: Infection of the implant ***(Case 1, 3, 6)***, aseptic cup loosening at the cement-graft interface ***(Case 7, 8)***, recurrent subluxation ***(Case 2)*** and excision of ectopic bone ***(Case 4)***. Biopsies were taken during these procedures with a Yanshini needle (diameter 4 mm) by the same surgeon who revised the cup ***(Figure 1)***. During the biopsy procedure diagrams were made

TABLE 1

Case	Age (years)	Gender	Side	Original diagnosis	Number previous revisions	Implant removed at revision	Implant placed at revision
1	85	F	L	OA	0	Gamma nail	Exeter THP
2	64	F	R	OA	1	Muller curved stem	Muller straight stem
3	64	F	R	RA	1	Charnley Muller THP	Charnley THP
4	55	M	L	SOA	0	Wagner double cup	Charnley THP
5	45	F	L	SOA	0		Exeter THP
6	76	M	L	OA	0	Muller straight stem	Charnley THP
7	66	F	R	RA	0	Muller curved stem	Exeter THP
8	77	M	L	SOA	0	Mac Kee Farrar cup	Charnley THP

Case	Type of defect	Type/amount of graft	Follow up (months)	Reason for taking biopsies	Type of biopsy specimen
1	Cavitary	Allograft 2 femoral heads	1	Infected THP	Whole reconstruction
2	Cavitary, dorsal rim	Allograft 1 femoral head	4	Reduction of luxation	Three core specimens
3	Cavitary	Allograft 1 femoral head	8	Infected THP	Two core biopsies
4	Cavitary, medial wall	Allograft 1 femoral head	9	Removing ectopic bone	Two core biopsies
5	Cavitary, dorsal rim	Allograft 3 femoral heads	15, 28	Sciatic nerve	Two core biopsies, whole reconstruction
6	Cavitary	Autograft	19	Infected THP	One core biopsy
7	Cavitary, medial wall	Allograft 1 femoral head	22	Mechanical failure	Two core biopsies
8	Cavitary	Allograft 2 femoral heads	72	Mechanical failure	Bone at graft-cement interface

Table 1
Summary of patient information

of the location of the biopsy needle Only biopsies that remained intact during the whole embedding procedure were used for evaluation In cases 2, 3, 4, 6 the cup remained in position during the biopsy procedure, which did not allow biopsies of the graft-cement interface Biopsies were taken in these cases from the cranial or caudal part of the reconstruction In one patient ***(Case 5)*** with secondary OA due to congenital hip dysplasia, the cup had been implanted at the original level of the tear drop with a voluminous acetabular graft during the first operation, thus bringing the centre of rotation about 5 cm more distally The patient subsequently developed sciatic nerve problems The sciatic nerve was released 15 months postoperatively, and 2 biopsies were taken Due to persistent complaints the hip was re-revised and a new reconstruction with a higher centre of rotation was created in order to release the sciatic nerve During this re-revision, the first reconstruction was removed which allowed histological inspection of almost the whole previous graft including the graft-cement interface

The specimens were fixed in phosphate-buffered (0 1 M, p 7 4) 4% formalin and embedded non-decalcified in methylmethacrylate Serial sections (7 μm thick) were stained with haematoxylin eosin and Masson trichrome staining to visualize osteoid Normal and polarized light were used to detect woven bone

RESULTS

Remnants of the graft could be clearly recognized on the basis of empty osteocyte lacunae In none of the core biopsy specimens could the host graft interface be found

No incorporation of the graft was found in the biopsy taken at one month ***(Case 1)*** Only dead bone trabeculae were present with avital remnants of bone marrow At the graft-cement interface a substantial interlock was found between graft and cement In 2 out of the 3 biopsies taken at 4 months, a front of new bone was penetrating the avascular graft At the revascularization front, which could be recognized on the basis of vital soft tissue, osteoid and woven bone formed on the original graft trabeculae and in the interstitial space ***(Fig. 2A and B)*** Also, there was local osteoclastic resorption of the graft

All of the specimens taken 8-28 months after revision showed different stages of graft incorporation At 8 and 9 months, various amounts of graft remnants were embedded in a new trabecular structure ***(Fig. 2C and D)*** The newly formed bone was woven Generally, there was an increase in the amount of woven bone towards the graft-cement interface, which suggested that after a time, woven bone was remodelled into lamellar bone In four specimens ***(Case 1, 5, 7 and 8)*** the graft-cement interface was present in the biopsy In case 7 and 8 no intact interface was present due to the loosening process, but there was only vital bone without remnants of graft In case 5 the interface was present over large areas of the reconstruction Locally vital bone was in direct contact with the cement, but at most locations a thin soft tissue layer interfaced with the cement

In the biopsies taken 15, 22 and 28 months postoperatively, remnants of the graft were extremely scarce ***(Fig. 2E and G)*** Most of the woven bone had been remodelled into lamellar bone with a normal structure and with vital, cell-rich medullary tissue Larger bone specimens were available from Case 8 because the whole reconstruction had to be moved to a higher centre of rotation All bone specimens from this patient showed normal lamellar bone with little or no graft remnants ***(Fig. 2H and J)*** Graft remnants and remnants woven bone were only present at locations where a very dense trabecular structure had formed, for instance on the margins of the new acetabular wall

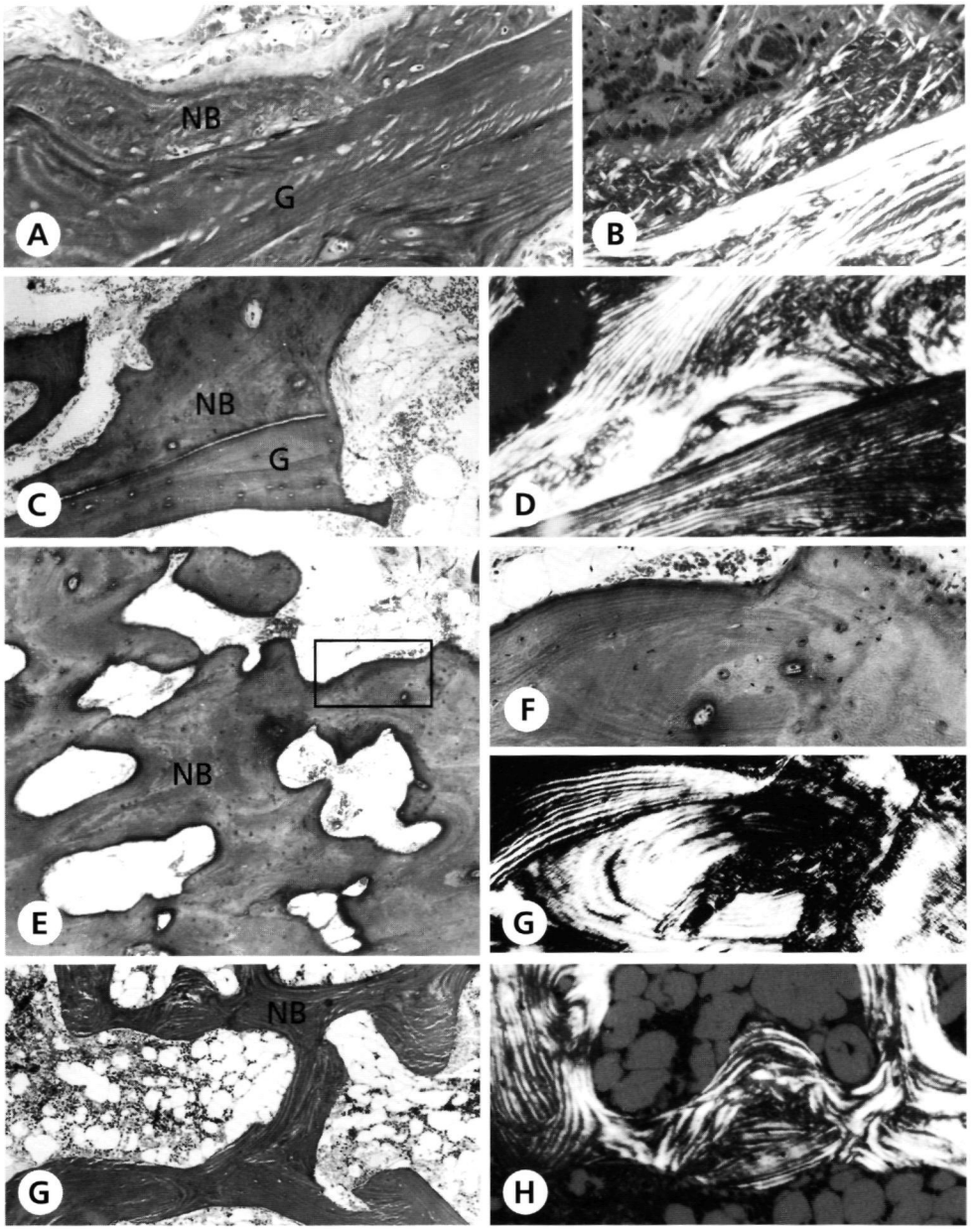

Figure 2

***A and B**: Human core biopsy four months after impaction grafting in the acetabulum. Note new bone formation (NB) on remnant of graft (G). **B** Same as **A** but with polarized light. Note woven bone formation. x140. **C** and **D** Eight months after impaction grafting. Graft (G) has been partially resorbed. New bone formation in the form of lamellar bone. x60 and x140, respectively. **E-G** Fifteen months after impaction grafting. Graft remnants are extremely scarce. Detail of boxed area shows vital (F) lamellar (G) bone. x35 and x140x, respectively. **H and J** Normal lamellar (J) bony structure with vital cell rich haemopoetic tissue (H) in specimen 28 months after impaction grafting. x60.*

DISCUSION

Biopsy specimens have limitations for the evaluation of all the processes involved in the graft incorporation process. In most cases only a limited area of the total reconstruction can be investigated and the histology of the medullary tissues may be compromised by the biopsy procedure. Furthermore, in the case of fixed cups that were not revised the biopsy specimen does not contain the cement-graft interface, or if the biopsies are of a mechanically failed cup, the histology of the cement-graft interface is compromised by the failure process. As a result an intact cement-graft interface was only present in one of the biopsy specimens (Case 5, 28 months).

In the specimen 1 month post-revision, no signs of graft incorporation were found. All specimens with a postoperative period of 8 months or longer showed a new trabecular structure, initially with woven bone that subsequently remodelled into lamellar bone. The bone in the specimens with a follow-up of 15 months or longer closely resembled normal trabecular bone, with only very few remnants of graft. Similar results were found in the proximal femur after impaction grafting [3,4]. The sequence of events was comparable to that previously observed in animal experiments in the goat [1,5,6,8].

In conclusion, our results indicate that impacted morsellized chip graft completely incorporates into a new trabecular structure.

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CHAPTER

THE REPEATED SAMPLING BONE CHAMBER: A new permanent titanium implant to study bone grafts in the goat.

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Laboratory Animal Science 47:401-406, 1997.

ABSTRACT

Our objective was to study various aspects of the bone allograft incorporation process under reproducible non-load bearing experimental conditions in larger animals For this purpose the Repeated Sampling Bone Chamber (RSBC) was developed
Our chamber is made of commercially pure titanium and provides for longitudinal bone or tissue ingrowth into a removable inner core Three chambers per animal were randomly implanted in the medial right and left tibiae of ten goats and harvested every eight week In experiment one, one chamber was filled with fresh-frozen structural allograft, one with fresh frozen chip allograft and one was left empty as control In experiment two, all chambers were left empty The results were evaluated by histomorphometry The clinical results of another 4 experiments are also presented to underpin the harvest principle

With this model 60 harvest operations have been conducted in the course of one year, with a mean of 5 0 ± 2 2 harvests per animal In experiment 1, more soft tissue ingrowth and osteoclasts were measured in the chambers containing allograft ($p<0.005$ and $p<0.03$ respectively) The bone ingrowth was scant, with no significant difference between the chip graft, structural graft and the empty control chamber (0 1 ± 0 2, 0 5 ± 0 2 and 0 6 ± 0 2 mm respectively) Thus, the bone graft showed neither osteoinductive nor osteoconductive properties in this unloaded situation Experiment 2 demonstrated consistant tissue ingrowth, independant of chamber localization
Our method allows for studying gradual tissue and bone ingrowth into bone grafts The inherent low bone ingrowth makes this model suitable to study bone inductive substances The repeated experiments in the same animals lowers the experimental intersample variability and also reduces the number of the large laboratory animals to be killed

INTRODUCTION

The amount of bone grafts used in bone-reconstructive surgery is rapidly increasing The available bone auto- and allografts may become insufficient to cover the demands in the future Furthermore, the use of bone grafts entails the risk of disease transmission and requires expensive testing for infectious agents The development of bone graft replacing materials and bone-growth stimulating factors therefore seems important
There are *in-vivo* models in which various materials and factors have been tested for their capacity in graft incorporation and bone healing [1,2] These experiments were conducted in small laboratory animals and because the rate and potential for bone repair appears to be inversely related to the size and age of the animal [3], these studies are remote from the human situation Also the amount of material that can be tested in these models is limited [1,2] Other *in-vivo* models in larger animals are not standardized with respect to the mechanical loading condition [4], an important factor in the bone healing process, so that the pure biological influences of the implanted materials are obscured

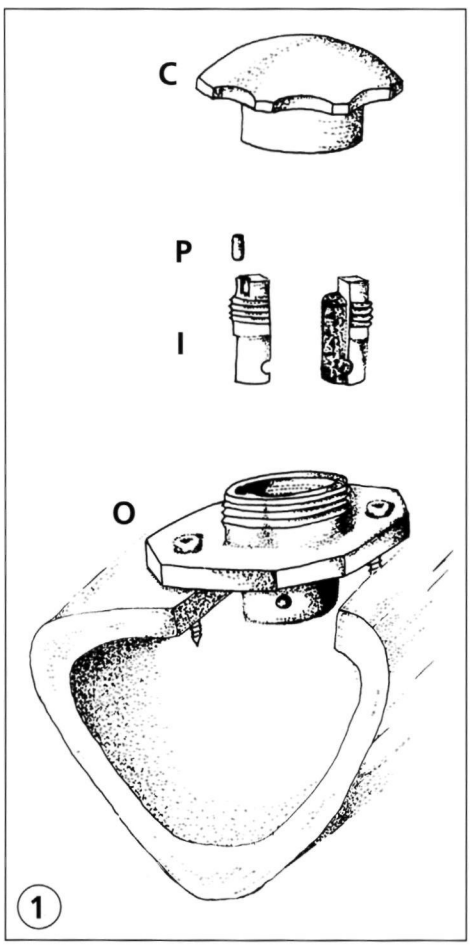
We adapted the bone chamber model used in rodents [2] for use in a larger vertebrate in order to investigate the *in-vivo* influences of bone processing and growth factors on tissue and bone ingrowth, implant resorption and incorporation under reproducible, non-load bearing conditions In this paper we discuss the properties of the bone chamber in relation to the results of two experiments

MATERIALS AND METHODS

Implant

The Repeated Sampling Bone Chamber (RSBC) is made from commercially pure titanium It consists of a cylindrical outer housing and a removable inner core, in which the

material to be investigated is placed ***(Fig.1)***. The outer housing is 6.7 mm in diameter and 10 mm in height. It has a closed bottom and is permanently fixed to the proximal medial tibia with two flanges and 2 cortical screws (2.7 mm x 12 mm). The inner core consists of 2 half cylinders (inner diameter 3 mm, height 7 mm), and can be screwed into the outer housing. The outer housing and the inner core both have two round in-growth-holes (diameter 1.5 mm), that are in direct contact with the tibial cortex ***(Fig. 2)***. When the chamber is assembled, a peg prevents rotation of the inner core in the outer housing, assuring a stable con-duit for invasive tissue and bone ingrowth. The chamber is closed with a screw cap.



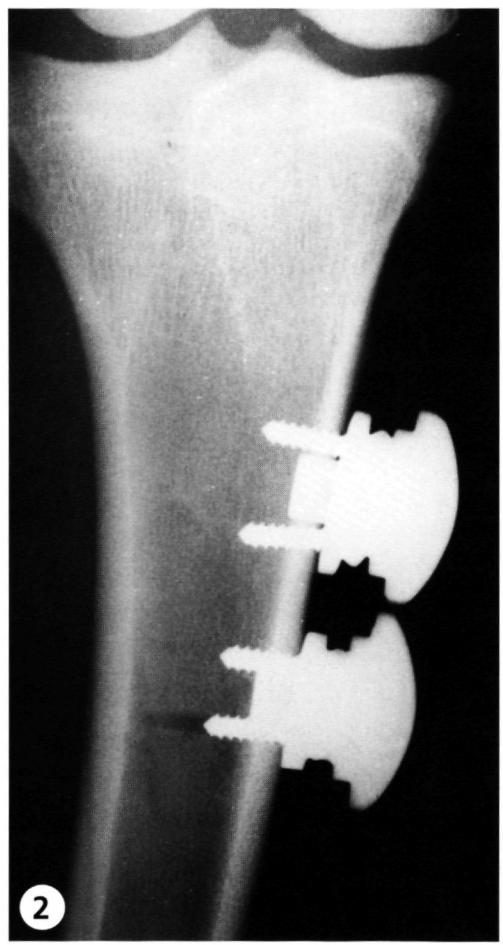


Figure 1
The Repeated Sampling Bone Chamber (RSBC). O = Outher housing, fixed to the proximal medial tibia with two cortical screws. I = Inner core, consisting of two halve cylinders that can be screwed into the outher housing. P = Security-peg, prevents rotation of the inner core in the outher housing. C = Mushroom-shaped screw cap. After osseointegration of the implant, the bone can grow into the chamber through the two ingrowth holes.

Figure 2
Roentgenological picture of bone chambers implanted at the medial tibial side.

Operation

Ten mature goats (Capra Hircus Sana) received each three chambers in the medial side of the proximal tibia, divided over the left and right side The side to receive two chambers was chosen by random The goats were anaesthetized with pentobarbital (Narcovet® 60mgr/ml, Apharmo) at a dose of 0 5 ml/kg body weight, intubated and maintained using halothane and oxygen in a semiclosed ventilation system The lower legs were shaved, washed, jodined and covered up with sterile cloths A curved incision was made in the skin and fascia over the medial side of the proximal tibia A drill-hole was made in the medial cortex with a 6 7 mm drill with diamond tipp connected to a saline solution irrigation system (Surgical Diamond Instruments® Scientific Developments, Munich), avoiding heat-induced necrosis of the periimplant bone After drilling the holes for the cortical screws, the outer housing was fixed to the tibia with small cortical screws The chamber is situated with its ingrowth holes in the longitudinal axis of the bone The inner core was screwed in, the peg placed and the implant closed with the screw cap In the tibia with two chambers, these were inserted at 10 mm distance from one another The facia and skin were closed separately After the implan tation procedure, the animals received subcutaneous ampicillin (Albupen LA® 100mg/ml, Mycofarm) 7 5 ml per dag for 5 days, but no medication was given after the harvesting operations Postoperatively, the animals were allowed unrestricted ambulation and free access to water and food

Experimental set up

Before starting the experiments, the empty chambers were harvested after an eight week postoperative healing period These so-called 'priming' specimens were not used for histomorphometry, but only served as control that the chamber seating allowed for tissue and bone-ingrowth All following harvest operations were performed at 8 weeks intervals

In experiment 1, the goats received fresh-frozen allografts from a non-relative donorgoat in two of the RSBC's The first chamber was filled with impacted chips, the second with a structural trabecular cylinder and the thirth chamber was left empty as control The grafts and the empty control were distributed over the chambers, so that all localisations had the similar incidence

In experiment 2 all three chambers were left empty The specimens were used to investigate the inter and intra animal variability of tissue and bone ingrowth
After these experiments, another four harvest operations for other experiments have been conducted in the group of ten goats In order to substantiate the repeated harvest potency of this model the clinical results of these experiments will be presented here

Graft preparation

The allografts were taken from the sternal bone of a donor goat, either as small chips or cylinders (size 3x7 mm) The graft material was cultured, packed in sterile bags and stored at -70°C until use Before implantation the grafts were thawed in saline

Histology

All specimens were fixed immediately after harvest in formaldehyde 4%, dehydrated in ethanol, embedded in plastic and sectioned longitudinally along the axis of the cylindrical specimen in 7μ thick slides Sections were processed for non-decalcified routine-histology (Haematoxylin-Eosine according to Mayer and Goldner-Masson trichrome) and enzyme histochemistry (Alkaline Phosphatase [AP] and Tartrate-Resistant Acid

Phosphatase [TRAP]) Histomorphometry was performed with a microscope connected to a computerized video digital table system (Videoplan, Kontron Bildanalyse GMBH, Germany) The sections were measured for area of tissue ingrowth (ATI in mm^2), area of bone ingrowth (ABI in mm^2), and width of the specimen (WS in mm), all with a microscope magnification of 25x Relative ingrowth variables were calculated for tissue ingrowth distance (ATI/WS) and bone ingrowth distance (ABI/WS) These variables represent the mean longitudinal ingrowth distance in millimeters into the inner chamber Osteoclast number was counted using a microscope magnification of 100x The reproducibility of the tissue measurements was 2 5 percent and of the cell measurements 5 percent

Statistical evaluation

Results of experiment 1 were tested with a two way ANOVA for the factors goat and type of implant in the chamber A simultaneous test to prove pairwise differences between the bone chamber contents was conducted according to the Bonferroni principle A Spearman correlation was calculated for the coherence between tissue ingrowth and osteoclast number Experiment 2 was tested with a three-way ANOVA for the factors goat, RSBC localisation and cortex thickness at the RSBC localisation

RESULTS

Clinical evaluation

After the 'priming' operation we have performed 60 harvest operations (6 experiments), with a mean of 5 0 ± 2 2 harvests per animal In experiment 1, the specimens of one goat were lost because of infection of the RSBC's In total six goats had to be replaced by new ones during the 60 harvest operations, all because of infection of the RSBC's Six goats maintained their RSBC successfully during all harvest operations Generally, all goats tolerated the bone chambers well, with good fixation of the implants over a period of 6-12 months without skin ulcerae or wound healing problems Radiographs demonstrated cortical thickening around the implants after 8 weeks, resulting in a evident covering of the ingrowth holes by cortical bone

Harvest evaluation

After the priming period (eighth weeks), tissue ingrowth had occurred in all of the chambers, however was constrained to fill up the empty space between the two ingrowth holes, with no tendency to further fill the chamber The amount of bone in the specimens varied between absence of bone tissue and the presence of a nearly uninterrupted bone layer between the two ingrowth holes

In both experiments 1 and 2, the specimens of two goats were intended for future immunohistochemical evaluation and therefore could not be processed for the routine histology Because in experiment 1, one goat was lost due to infection, the specimens of seven goats were available for histological and histomorphometric analysis

The specimens of experiment 1 showed consistent soft tissue and variable bone ingrowth The empty control chambers contained fibrous tissue with macrophages, and sometimes woven bone with a few osteoclasts In the graft material more macrophages were present and on the surfaces of the dead bone graft abundant TRAP-positive osteoclasts were found The ingrowing bone appeared to be formed by membraneous ossification ***(Fig. 3)*** and no cartilage was detected The factor RSBC content appeared significant for tissue ingrowth distance ($p<0\ 005$) and osteoclasts number ($p<0\ 03$) For bone ingrowth distance the factor goat was significant ($p<0\ 01$), but not RSBC content The chambers with the chip and structural grafts had more tissue ingrowth than the empty

controls (3.2 ± 0.5 mm, $p<0.05$ and 4.4 ± 0.5 mm, $p<0.01$ respectively) ***(Table 1)***. The bone ingrowth was scant, with no significant difference between the chip graft, structural graft and the empty control chamber (0.1 ± 0.2 mm, 0.5 ± 0.2 mm and 0.6 ± 0.2 mm respectively). Compared to the control chambers the grafted chambers also contained more osteoclasts ($p<0.02$). The correlation coefficient for tissue ingrowth and osteoclast number was 0.84 for chip graft, 0.60 for structural graft and 0.52 for the control tissue.

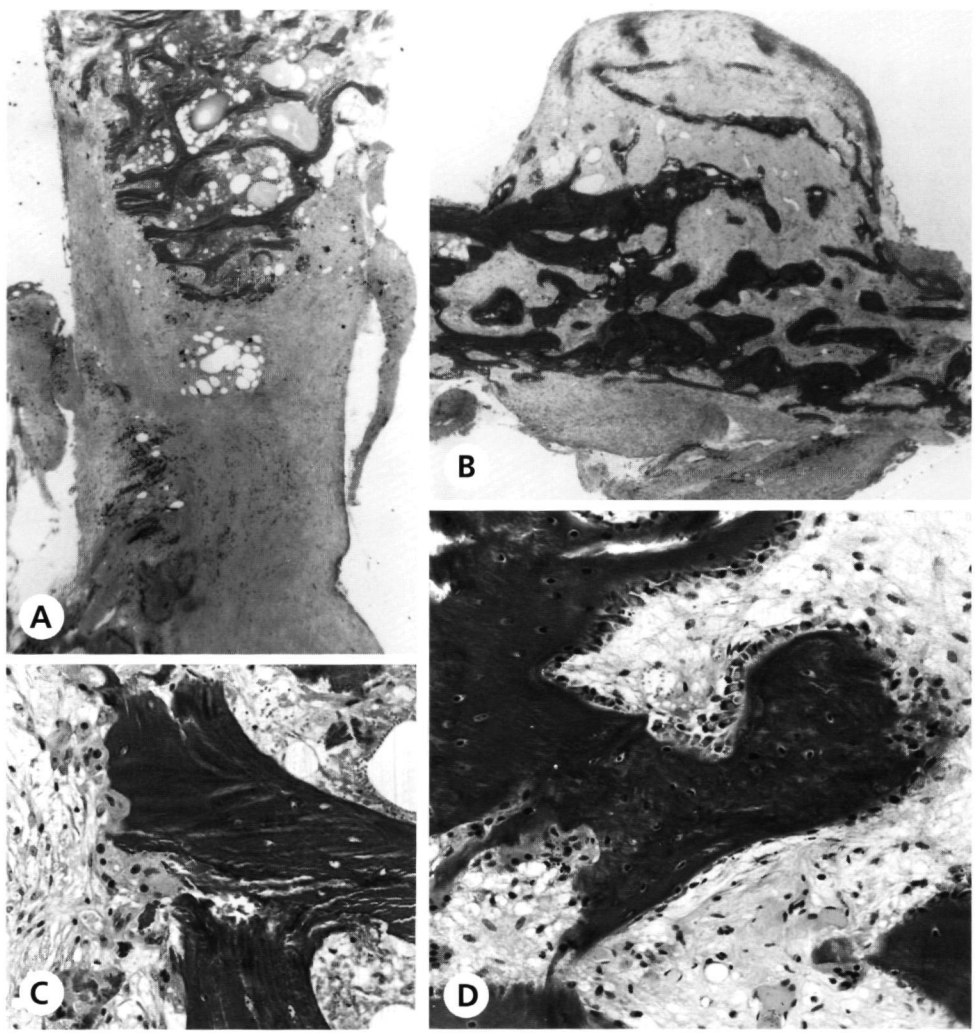


Figure 3
A) *Fresh-frozen allograft with osteoclastic bone resorption at 8 weeks. A sharp border is seen between the ingrowing tissue and the graftmaterial. At the bottom there is membranous bone formation. A detail of osteoclastic graft resorption is demonstrated in part C [Hematoxyline-Eosin, x12].* ***B)*** *Tissue from the 'control' RSBC harvested at 8 weeks. Bone ingrowth from the two ingrowth holes. A detail of the immature woven bone is given in part D. There is no tendency to fill the whole chamber with new bone [Hematoxyline-Eosin, x12].*
C) *Detail of osteoclastic graft resorption [Hematoxyline-Eosin, x100].* ***D)*** *Detail of membranous ossification [Hematoxyline-Eosin, x100].*

In experiment 2, none of the experimental factors goat, chamber position and cortical thickness at the bone chamber localisation were significant The amount of tissue ingrowth and osteoclast number varied less than the amount of bone ingrowth The coefficient of variation for tissue ingrowth was 0 3, for osteoclast number was 0 8 and for bone ingrowth this was 3

TABLE 1

	EXPERIMENT 1			EXPERIMENT 2				
	CHIP	CONT	STRU	BC<2	BC>2	A	B	C
TID	3.2*	1 7	4.4 ■	3.0	3.2	2.3	2.8	2.4
	0 5	*0 5*	*0 5*	*0 5*	*0 4*	*0 9*	*0 7*	*0 7*
BID	0 1	0.6	0 5	0.2	0.6	0.0	10.4	0.1
	0 2	*0 2*	*0 2*	*0 2*	*0 2*	*0 0*	*40 9*	*0 3*
OCLAST	156.0#	52.5	160.0#	107.0	138.8	29.3	39.0	43.7
	27 7	*27 7*	*26 9*	*29 6*	*22 3*	*26 4*	*32 6*	*36 7*

Notes **TID**=tissue ingrowth distance in millimeter **BID**= bone ingrowth distance in millimeter **OCLAST**=osteoclast number **BC<2**=bone chamber located at cortex thinner than 2 mm **BC>2**=bone chamber located at cortex thicker than 2 mm **STRU**=structural allograft **CHIP**=impacted chip allograft **CONT**=control empty chamber **A**= proximal chamber of two **B**=distal chamber of two **C**=single chamber ■ p<0 01 # p<0 02 * p<0 05

Table 1
Histomorphometric results of the bone chamber specimens ***Mean*** *and SD*

DISCUSSION

When investigating the healing response of bone defects, filled with bone grafts or biomaterials, it is very important to standardize the main influential factors in the restoration process Biological influences derive for instance from several growth factors, signalling proteins and bone marrow cells Another important influential factor is the mechanical loading condition [5] In our bone chamber model, the invasive tissue grows into the chamber towards the investigated material perpendicular to the loading axis of the tibia By situating the bone graft exterior to the tibial loading axis, it is possible to study biological factors in the bone graft incorporation process in the absence of interfering mechanical influences

We experienced few clinical complications, which were solely caused by infections The chambers were well fixed in the tibial bone and no skin healing problems occurred When taking into account that the skin will get more vulnerable after several reoperations, the complication number of these experiments is satisfactory Commercially pure titanium is well tolerated as a permanent implant [6] Because of the use of this material and the good perioperative conditions, repeated harvesting in the same experimental animals was possible

The quantitative analysis of the RSBC experiments is based on histomorphometry of the tissue response to the implanted material The tissue ingrowth and osteoclast number were increased by a bone graft, but were independant of cortical thick ness at implantation side and bone chamber position However, bone ingrowth varied considerable The presence of the cortical bone layer, sealing of the chamber ingrowth-

holes, seemed not to have affected the strength of the healing response, but could have had some influence on the direction of the tissue differentiation in the individual goat

There was a variation in response between the animals, resulting in a rather wide-spread distribution of the histomorphometric measurements Because we used three chambers per animal, the inter-animal variation could be eliminated by using an intra-animal control The high standard deviation and the small number of experimental animals jeopardize the discriminating power of the graft experiment By increasing the number of animals or implanting more active materials this problem might be overcome The repeated sampling of the same investigated material will contribute even more in this respect Another advantage of the repeated sampling is the reduction of the number of expensive and large laboratory animals that have to be killed

The two types of allografts revealed comparable cellular reactions, with abundant macrophages and osteoclasts However, the response varied between the different goats This suggests that the allograft, although deriving from only one donor goat, was not equal immunogenic in every host goat This could be based on an effect of the major histocompatibility complex antigens or the presence of necrotic tissue [7]

The low bone ingrowth in the bone grafted chambers indicates that no clinically important bone growth stimulatory activity is being released from this type of bone graft Stimulation of bone ingrowth could derive from growth factors, like Bone Morphogenetic Proteins (BMPs), that are released from the bone matrix during resorption BMP is present in minute amounts in natural bone, whereas activity for progenitor proliferation and differentiation can only be expected with pharmacological amounts of micromolar concentrations [8] The low initial bone ingrowth makes this bone chamber model suitable to study potentially bone growth stimulatory agents combined with generally applied bone graft materials

In our specimens also no signs of osteoconduction were observed However, in animal experiments, in which impacted chip grafts have been implanted in the femur [9] and acetabulum of the goat [10], the chips served as a scaffold for new bone apposition It appears that, for this phenomenon to occur, mechanical loading may be a prerequisite, generating micromotion between the chips The structural graft, on the other hand, constitutes a stable construction in hip reconstructive surgery, with no micromotion inside the graft Only marginal graft incorporation has been observed with this type of graft [11] The absence of mechanical loading onto the structural graft specimens in our experiment probably explains for the different histological pattern compared to the human retrieval studies

Our bone chamber model allows for bone and tissue ingrowth measurements, whereas in some comparable animal models the chambers are always entirely filled with bone tissue within a short healing period The 'Bone Harvest Chamber' is implanted in cortical bone of rabbits to study small bone healing specimens [1] Another model, using the 'Analytic Bone Implant', aims at investigating the restoration response of trabecular bone [12] Because in both models the bone recovery is already maximal within a short time-span, the designs are not appropriate to study bone healing stimulative materials and soft tissue responses

In conclusion, the Repeated Sampling Bone Chamber can contain sufficient amounts of bone graft to study tissue and bone ingrowth under controlled conditions The chamber is designed for repeated experiments in the same animal, which lowers the intersample variability in the experiment and the mortality number of the laboratory animals

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CHAPTER

GROWTH FACTORS IN THE INCORPORATION OF UNLOADED BONE ALLOGRAFTS IN THE GOAT.

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Clinical Orthopaedics and Related Research (submitted)

ABSTRACT

Revision of a failed total joint replacement often demands bone grafting methods to restore a deficient bone stock However, impaired graft incorporation can be the result of inadequate host or graft properties The stimulation of bone healing with growth factors, could provide a new approach in dealing with this problem We used the Repeated Sampling Bone Chamber (RSBC) in the goat to investigate the properties of bone allografts enriched with Transforming Growth Factor-ß (TGF-ß2), recombinant human Bone Morphogenetic Protein-2 (rhBMP-2) and basic-Fibroblastic Growth Factor (b-FGF) under unfavourable vascular and non-loaded conditions

Ten goats had three RSBC each in the medial proximal tibia All chambers received processed bone allograft either with or without a growth factor The time period between chamber harvests was 8 weeks Experiment 1 0, 1 or 10 μg of TGF-ß2 Experiment 2 0, 1, or 5 μg of rhBMP-2 Experiment 3 0, 40 or 200 ng of b-FGF The specimens were analysed histomorphometrically for the amount of soft tissue ingrowth, bone ingrowth and the number of osteoclasts

Three goats had to be replaced because of infection of one of their chambers In all specimens, a resorption front was growing into the graft followed by fibrovascular tissue and in some instances bone In the 5 μg rhBMP-2 specimens larger amounts of soft tissue and woven bone were present ($p=0.05$ and $p=0.02$ respectively), whereas 10 μg of TGF-ß2 decreased the amount of tissue and bone ingrowth ($p=0.05$) Also 200 ng of b-FGF had a negative effect on soft tissue formation ($p=0.03$), and instead vascular elements with erythrocytes were abundant The number of osteoclast was higher in the rhBMP-2 5 μg specimens ($p=0.04$) In a clinical case with absence of a good perigraft vascularisation and loading, rhBMP-2 could probably have the most effective influence on bone graft incorporation

INTRODUCTION

Morsellized bone grafting techniques at the acetabular [51] and femoral side [21] have been used to restore inadequate bone stock during revision surgery However, impaired bone graft incorporation has been found at stress-shielded areas behind protrusio cups (Buma, unpublished results)

Bone allografts are thought to have osteoinductive and osteoconductive properties, however these capacities seem to be graft type dependent Particularly massive cancellous bone grafts demonstrate only marginal osseointegration at the graft-host interface with limited osteoconduction and incorporation [24 15] Histological studies of the morsellized bone allografts on the other hand reveal graft incorporation at 8 months [10], demonstrating osteoconduction of new woven bone along dead bone graft fragments and remodelling into a new vital trabecular structure Recently, Aspenberg et al [3] demonstrated that the process of osteoconduction in the morsellized graft is under influence of bone proteins present in bone matrix This may suggest that bone ingrowth is partly directed by osteoinductive activity

The stimulation of bone healing by growth factors could provide a new approach in dealing with impaired bone graft incorporation due to unfavourable graft or host conditions Various *in-vivo* studies have demonstrated positive effects of externally applied growth factors on bone graft incorporation and bone healing in animals [13 14 66 29] In some long bone defect models however, the effect of mechanical loading on the incorporation process was not excluded Other studies using relatively unloaded transplantation sides, have optimal vascular circumstances in active remodelling trabecular bone, muscular tissue or local periosteal tissue [3 4 5 7 38 52] The most attractive utilization of growth factors would be the application under unfavourable circumstances like inadequate loading, inferior bone graft or host bone qualities

In this paper we describe the osteoinductive and osteoconductive properties of processed bone grafts (demineralized bone graft, defatted bone graft) and growth factors (Transforming Growth Factor-ß2, TGF-ß2, human recombinant Bone Morphogenetic Protein-2, rhBMP-2, and basic-Fibroblastic Growth Factor, b FGF), under unfavourable vascular and non-loaded circumstances in a bone chamber model in the goat

MATERIALS AND METHODS

Repeated Sampling Bone Chamber

The Repeated Sampling Bone Chamber (RSBC) was developed to study various aspects of the bone allograft incorporation process under reproducible non-loaded experimental conditions in larger animals [31] The chamber is made of commercially pure titanium and consists of a cylindrical outer housing with a removable inner core The chamber is permanently fixed to the proximal medial tibia There are two round ingrowth openings at that end of the chamber which is in direct contact with the tibial cortex These openings serve as a one-sided conduit for invasive tissue and bone ingrowth towards the graft material inside the chamber

Operation

Thirteen mature goats (Capra Hircus Sana) received three RSBCs each, implanted in the medial side of the proximal tibia, divided over the left and right side The side to receive two chambers was chosen randomly During the harvest operations three goats had to be replaced by new ones due to infection of one of their implants This means that a number of ten goats was available for each experiment The goats were anaesthetized with pentobarbital (Narcovet® 60 mgr/ml, Apharmo) at a dose of 0 5 ml/kg body weight Under aseptic conditions, a drill-hole (6 7 mm) was made in the medial cortex of the proximal tibia with a hollow diamond tipped drill (Surgical Diamond Instruments® Scientific Developments, Munich) Heat-necrosis of the cortical bone was avoided by connecting the drill to a saline solution irrigation system The chamber was situated with its ingrowth holes in the longitudinal axis of the bone and fixed to the tibia with small cortical screws The inner core, containing the type of prepared bone graft according to the experimental scheme, was screwed in and the implant closed with the screw cap During the harvest operations, only the inner core was removed and the graft material was removed, whereafter new grafts were placed in the bone chamber The harvest operations were performed every eight weeks Only one time, after the implantation procedure of the titanium implant, the animals received subcutaneous ampicillin (Albupen LA® 100 mg/ml, Mycofarm) 7 5 ml per dag for 5 days No medication was given after the harvesting operations Postoperatively, the animals were allowed unrestricted ambulation and free access to water and food

Growth Factor solution and Graft preparation

The allografts were taken from the sternal bone of a donor goat as cylinders (size 3x7 mm) The graft material was cultured, packed in sterile bags and stored at -70°C until use

De-Fatted freeze dried allograft Bone Matrix (DFBM): the grafts were defatted in methanol/chloroform 1 1 overnight, rinsed three times for 30 minutes with methanol and three times for 30 minutes with sterile water Thereafter the graft material was freeze dried overnight and stored in sterile containers at 4°C

Demineralized allograft Bone Matrix (DBM): the grafts were demineralized in 0 6 N HCl for 24 hours, rinsed 5 times for 30 minutes with sterile water, freeze dried overnight and stored as above

Inactivated Demineralized Bone Matrix (IDBM): After the demineralization procedure the

grafts were further inactivated by 16 hours of 4N Guanidine/50 mM Tris The grafts were rinsed 5 times for 30 minutes with sterile water, freeze dried overnight and stored as above
BMP-solution: rhBMP 2 was diluted in sterile Phosphate buffered Saline (PBS), and transferred to small cups containing 0,33 or 167 μg/ml of rhBMP-2 These were stored at -80°C until use Purity and biological activity were ensured with *in-vitro* assays performed at Genetic Institute (Genetics Institute, Inc, Andover, Massachusetts, USA) The Inactivated DBM was placed in the PBS solution with or without rhBMP-2 overnight The solution was totally resorbed by the graft, leading to an expected amount of about 0, 1, or 5 μg of rhBMP-2 [29 65] The DBM was also rehydrated overnight in PBS without growth factor
TGF-solution: TGF-ß2 was mixed in sterile carboxy-methyl-cellulose (CMC) 3° and transferred to small cups with 0 μg/ml, 50 μg/ml or 500 μg/ml of TGF ß2 The cups were
60 stored at -80°C until use Carboxymethyl-cellulose was chosen as a carrier for TGF-ß2 according to studies of Aspenberg and Lohmander [2] and Aufdemorte et al [5] The defatted allograft was placed in the CMC overnight with an uptake of approximately 20 μl, leading to an expected amount of 0, 1 and 10 μg of TGF-ß2 per graft [5 49]
b-FGF-solution b-FGF was mixed in 1% sodiumhyaluronate gel (Healon®, Pharmacia AB, Sweden), with concentrations of 0, 2 5 or 12 5 μg/ml of b FGF Hyaluronate was chosen as a carrier for b-FGF according to the study of Wang and Aspenberg [60] The cups were stored at -80°C until use The defatted graft was placed in the gel overnight with a gel-uptake of the graft of approximately 16 μl, leading to an expected amount of 0, 40 and 200 ng of b-FGF per graft [60]

Experimental design

The grafts were distributed over the three chambers, so that a similar amount of goats had each possible localisation of each growth factor concentration
Experiment 1 Defatted allograft with 0, 1, and 10 μg TGF-ß2 in 10 goats
Experiment 2 Inactivated DBM with 1 and 5 rhBMP 2 μg in 5 goats each and Inactivated DBM controls and DBM in 10 goats
Experiment 3 Defatted allograft with 0, 40 and 200 ng b-FGF in 10 goats

Histology and histomorphometry

After harvest, specimens were fixed in 4% formaldehyde, dehydrated in ethanol, embedded in plastic and sectioned non-decalcified along the long axis of the cylindrical specimen in 7μ thick slides Adjacent sections were stained for routine-histology (Haematoxylin-Eosine according to Mayer and Goldner-Masson trichrome) and enzyme histochemistry (Tartrate-Resistant Acid Phosphatase [TRAP]) Semi automatic histo morphometry was performed using a microscope connected to a interactive computeri zed video digital table system (Videoplan, Kontron Bildanalyse Oberkochen, Germany) Three sets of sections from the middle of the specimen at 200 μm distance, were used for analysis The sections were measured for area of tissue ingrowth and area of bone ingrowth, all with a microscope magnification of 25x The mean ingrowth distance was calculated by dividing the area of soft tissue and bone ingrowth with the width of the specimen Osteoclast number was counted using a microscope magnification of 100x
The results of the ingrowth distances were tested for significance using a 2 way ANOVA for each experiment for the factors goat and growth factor dose

RESULTS

Clinical evaluation

During the experiments 3 goats had to be replaced by new ones, because of infection or aseptic loosening of one of their bone chambers Overall, the goats tolerated the

bone chambers well, with good fixation of the implants over the experimental period for at least 32 weeks. No skin ulcera or wound healing problems have been observed in any goat The specimens of one goat in the TGF-ß2 group were lost due to inadequate processing, leaving the specimens from 9 goats for evaluation.

Histology

In all experiments, a cell rich mesenchymal tissue, was growing from the ingrowth openings into the graft and formed a resorbing front into the graft material. Large numbers of osteoclasts were present at the ingrowth frontier. No polymorphonuclear or plasma cells were present. In the highly cellular and vascular tissue of some specimens, ossification areas developed in the neighbourhood of the ingrowth openings in the absence of any cartilage ***(Fig. 1A)***. In the DBM specimens large numbers of TRAP-positive osteoclasts were present on the graft surfaces ***(Fig 1B)***. Only in the high dose rhBMP-2 specimens, bone apposition occurred directly upon the graft remnants ***(Fig. 1C)*** and compared to all other specimens, large areas of immature bone sprouts with active bone formation were observed ***(Fig. 1D)***. The high dose TGF-ß2 specimens revealed high numbers of foreign body giant cells ***(Fig 2A)***, and the amount of mesenchymal tissue ingrowth was limited ***(Fig 2B)*** The most prominent findings in the b-FGF-containing specimens were the abundance of vascular elements with erythrocytes, which seemed to function as 'condensation elements' for membraneous bone formation ***(Fig. 2C+D)***.

THE INFLUENCE OF GROWTH FACTORS

TABLE 1

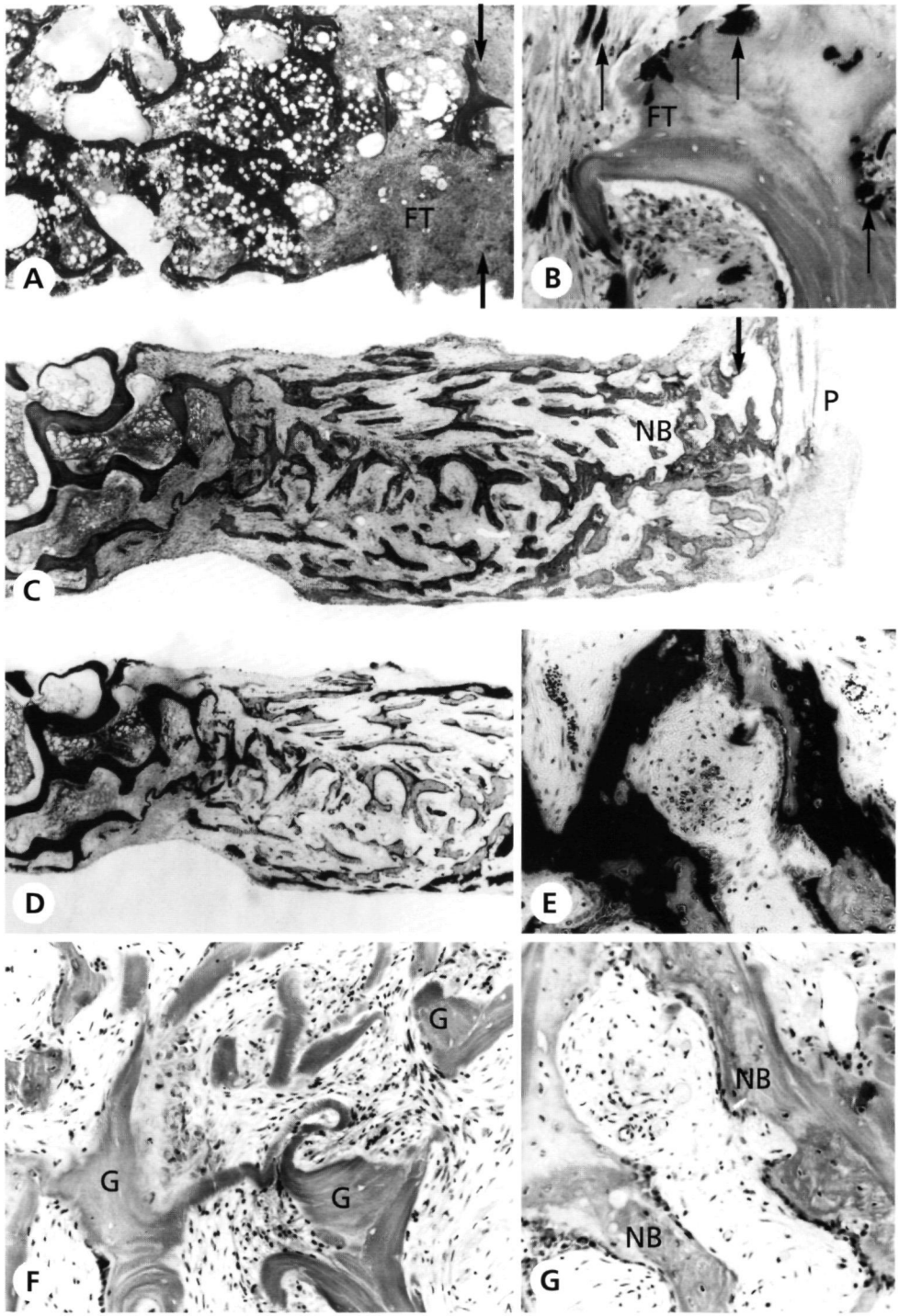
Experiment	GF	Goat (#)	Dose (ug or ng)	Soft tissue (mm)	Bone ingrowth (mm)	Osteoclasts (#)
1.	DFBM	9	0	3.9 ± 1.9*	0.4 ± 0.6*	151 ± 96
	TGF-ß2	9	1	2 8 ± 1.0	0	194 ± 83
	TGF-ß2	9	10	2.4 ± 1.0	0	188 ± 105
2.	IDBM	10	0	3.2 ± 0.9	0.01± 0.03	100 ± 53
	RhBMP-2	5	1	3.2 ± 0.8	0.1 ± 0 2	170 ± 145
	RhBMP-2	5	5	4.4 ± 1.8*	1.7 ± 2.0**	249 ± 102***
	DBM	10	0	2.6 ± 0.9	0.1 ± 0.1	340 ± 159****
3.	DFBM	10	0	4.3 ± 1.2	0.1 ± 0.4	118 ± 90
	b-FGF	10	40	4.2 ± 1.4	0.2 ± 0.4	94 ± 72
	b-FGF	10	200	3.0 ± 0.7▶	0.1 ± 0.2	116 ± 56

Notes Mean and standard deviation of soft tissue ingrowth, bone ingrowth and number of osteoclast GF (growth factor or treatment), IDBM (Inactivated allograft Demineralized Bone Matrix), DBM (Demineralized allograft Bone Matrix), DFBM (De-Fatted freeze dried allograft Bone Matrix)
*p=0 05, **p=0 02, ***p=0 04, ****p=0 001, ▶ p=0 03

Table 1. *Histomorphometric results*

Histomorphometry

High dose of rhBMP-2 produced more soft tissue ingrowth with subsequent graft resorption (p=0.05) and bone ingrowth (p=0.02) compared to its controls and rhBMP-2 at 1 µg ***(Table 1)***. The specimens with a high concentration of rhBMP-2 or consisting of DBM revealed larger amounts of osteoclasts as compared to the rhBMP-2 at 1 µg and


A
FT
B
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C
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D
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G
G
G
NB
NB

Inactivated DBM specimens (p=0.001) ***(Table 1)***. High concentration TFG-ß had a negative influence both on tissue and bone ingrowth as compared to the defatted allograft (p=0.05), but no influence was found on the number of osteoclasts ***(Table 1)***. High dose of b-FGF induced a decrease in soft tissue ingrowth (p=0.03), but b-FGF appeared to have no influence on the number of osteoclasts ***(Table 1)***.

DISCUSSION

Osteoinduction, i.e. differentiation of mesenchymal tissue into woven bone, has been found to occur under influence of several growth factors [34,41]. Osteoconduction has been defined as a more passive effect of a bone graft or tissue, able to serve as a scaffold for woven bone apposition. It had recently been demonstrated that this process is not as passive as assumed, but stimulated or facilitated by proteins present in bone matrix [3].

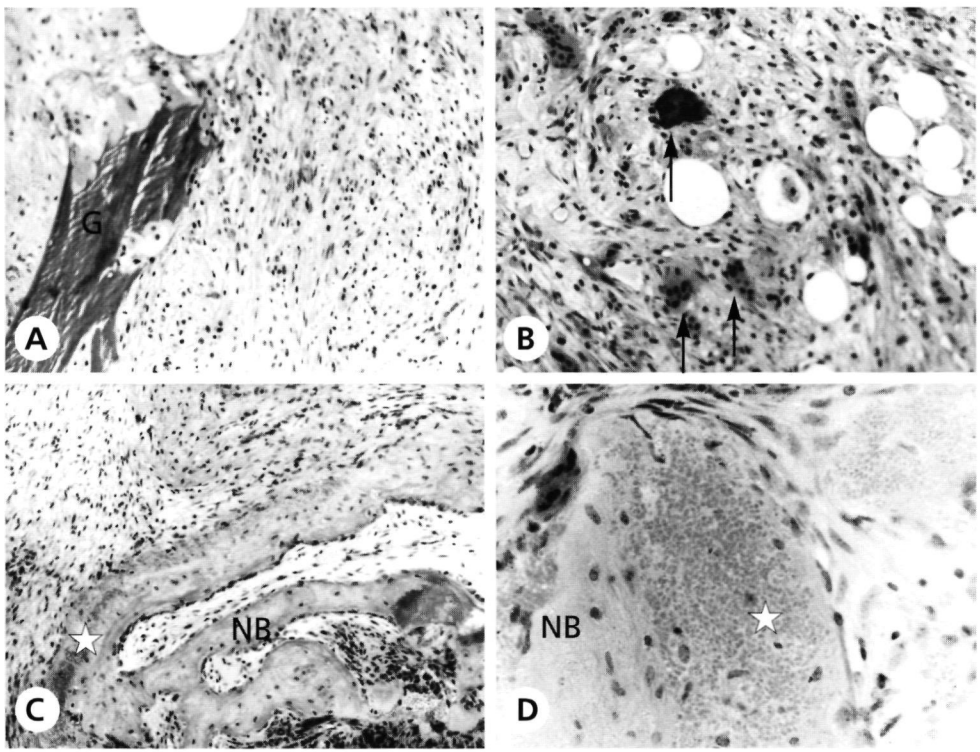

Figure 2. ▲
A and B. *TGF-ß specimen with abundant graft resorption activity (G). The ingrowing fibrous tissue is rich in multinucleated foreign body giant cells (arrows) (TRAP, x150).* ***C and D.*** *b-FGF specimen with new bone formation. Abundant vascular elements and erythrocytes (asterisks) seem to function as 'condensation elements' for membranous bone formation (NB) (Goldner,* ***C:*** *x150,* ***D:*** *x400).*

◀ **Figure 1.**
A and B. *Cell rich fibrous tissue (FT) with large numbers of TRAP-positive osteoclasts (arrows) present at the ingrowth frontier (****A:*** *HE, x17,* ***B:*** *TRAP, x140).* ***C.*** *Overview of rhBMP-2 specimen. Bone apposition occurred directly upon the graft remnants in the high dose rhBMP-2 specimens. Large amounts of immature bone (NB). The bone grows from the bottom of the chamber (arrow) into the graft (G). (HE, x17).* ***D and E.*** *Overview of same rhBMP-2 specimen with abundant bone remodelling activity (Goldner x17). Large areas of immature bone which starts to mineralize (Magnification of part of Fig. D, Goldner, x150).* ***F and G.*** *New bone apposition (NB) on graft remnants (G) in rhBMP-2 specimen. (HE, x150).*

In our model however, the endogenous growth factors in the DBM specimens did not positively influence the ingrowing mesenchymal tissue, neither through osteoinduction nor conduction.

Urist was the first to report the use of decalcified implants for ectopic induction of skeletal tissue [55,56]. DBM induces endochondral osteogenesis at ectopic sides in rodents [55], in non-union models [1,8,17,19], at extraskeletal sides in primates [48] and has also been applied for human purposes [40,54]. The bone morphogenetic proteins, in bone associated with the mineral phase and organic matrix, have been found to be responsible for the bone-induction phenomenon. In several non-rodent animal models however, DBM proved ineffective in healing diaphyseal defects [25,50] and inducing ectopic bone formation [2]. The clinical study of Toriumi et al.[53] reported an unacceptably high degree of resorption of DBM implants.

The demineralized bone matrix in our experiment elicited major numbers of osteoclasts compared to the inactivated DBM. The reason for this difference is not known The removal of the mineral matrix might have contributed to the recruitment of osteoclasts in an unknown manner Demineralization might also have deblocked antigenic proteins which could have give rise to an antigenic reaction, but this seems not so likely in the light of the low antigenicity of the bone matrix itself. This condition could acount for the suppression of all potential inductive activities in our DBM specimens. Although the antigenic differences in rodents probably are less than in higher vertebrates, due to use of outbred animals of the same strain [1,22], this can not fully explain our observation, because satisfactory results have been obtained in higher vertebrates including human. Other factors contributing to osteoinductive properties are the amount of surrounding mesenchymal cells, contact between the host material and bone-inducing surfaces and vascularity, so that the barren peri-graft environment in the bone chamber may have been detrimental. Another very important factor in the tissue response in this bone-chamber, may be the total lack of mechanical stimulation of the ingrown tissues in the direction of osteoblast precursors.

RhBMP-2 was the only growth factor in our experiments that increased bone ingrowth. The BMPs are known to stimulate mesenchymal stem cells to differentiate into osteoblastic and chondroblastic lineages [11,41,57,64]. Although the effects of BMPs depend on the osteogenic competence of the recipient, the site of implantation is also very important. Intramuscular implants have shown the highest activity in rodents [62], both for demineralized bone matrix and BMP-enriched grafts. Also in non-rodents, BMPs have proven efficient for bone healing [18,20,29,35,42,46], and also at extraskeletal sides [4,41].

Recently, an *in-vitro* study demonstrated that rhBMP-2 also stimulates bone resorption through both direct stimulation of osteoclast formation and activation of mature osteoclasts [27]. This observation is in accordance with our findings of larger numbers of osteoclasts and more implant resorption in the high dose rhBMP-2 treated grafts.

The recombinant BMPs have intact bone inducing capacities but need special carriers in order to maintain their activity at low doses [48,58]. Functional carries for BMPs include collagen matrix, demineralized bone matrix and various synthetic polysaccharide matrices [36,66]. The function of the carrier matrix is to immobilize the bone inducing protein at a particular side for a sufficient amount of time to allow bone induction to occur. The BMP-soaked inactivated demineralized bone matrix was able to induce a local process of woven bone formation under non-loaded and poor vascular circumstances.

In our study different carriers have been used for the different factors. Demineralized bone matrix, as well as carboxymethylcellulose and sodiumhyaloranate gel are all known as vehicles for local delivery of growth factors without disturbing the

intrinsic biological function of the substances [2 5 36 60] In order to be able to compare our results with other publication, we chose not to use one single carrier for all our experiments, but apply the most common used carrier for the specific growth factor

No differences in biological activity concerning the bone inductive properties and chondrogenesis have yet been determined between the TGF-ß1 and TGF-ß2 species [9 48] The results in our experiment are therefore discussed in relation to general TGF-ß characteristics and biological activities

TGF-ß appears to be a potent stimulator of chemotaxis of osteoblasts [44 45], and stimulator of the osteoblast proliferation [37] In-vivo studies of TGF-ß have demonstrated increased bone formation parameters in calvarian defects treated with single local TFG-ß application in rat, mouse and rabbits [7 26 43 28] Implant gap models with titanium and hydroxyapatite surfaces in the metaphysis of dogs also demonstrated enhanced bone growth onto the implants treated with TGF-ß with increased osteoprogenitor cells and matrix production [33 52]. In bone chambers implanted in the metaphysis in monkeys, containing similar TGF-ß concentrations to our experiment, there was no effect on the amount of ingrown bone, but the new bone showed increased osteoblastic activity [5] Subcutaneous implantation of TGF-ß however, did not result in bone formation but elicited a local fibrous cellular response [48] A bone chamber model similar to ours also demonstrated an inhibitory effect of TGF-ß on bone ingrowth [61] Although comparable amounts of TGF-ß have been applied in the cited papers, the difference in results may be explained by the localisation of experimental implantation sides

The TGF-ß enriched bone grafts displayed significant less tissue ingrowth and graft resorption compared to the defatted bone graft alone In-vitro studies described inhibition of bone resorption by TGF ß due to suppressing the formation and activation of bone-resorbing cells [9] In-vivo studies, describing an inhibitory effect of TGF-ß on bone healing, speculate about a difference in sensitivity and response to TGF-ß depending on the type of bone at the defect [32] As TGF ß displays pluriform actions and interactions with growth factors and cells, a clear explanation for the supressive action of TGF-ß in our experiment can not be given

Bone graft incorporation has been enhanced by b-FGF in bone chambers [59 60] In our study however, the highest b-FGF concentration decreased the amount of tissue ingrowth and did not affect the amount of bone ingrowth, despite the use of comparable concentrations to the forementioned studies b-FGF is a mitogen for chondroblasts and osteoblasts rather than an inducer of differentiation [23 41], so that increased tissue ingrowth, if not more bone, could have been expected Fact is that the chamber used in the rat experiments has a smaller ingrowth distance between the two ingrowth openings compared to the RSBC (1 0 mm versus 1 6 mm) These implant specific differences will probably play a critical role in the experimental outcome and therefore make a direct comparison of the b-FGF results obtained with the rat model with our b-FGF findings difficult

The specimens treated with the low dose of b-FGF demonstrated large amounts of vascular elements with erythrocytes and platelets The latter seemed to function as condensation centre for membraneous ossification b-FGF is known to be a potent stimulator of vascularization [6 16 39], with high concentrations of b-FGF present in the alpha granules of the platelets [41] Perhaps factors released from the platelets together with b-FGF create a more optimal environment for membraneous ossification

Overall, there are at least three classes of factors affecting bone formation, those that are mitogenic, those that induce differentiation of cells of the osteoblastic lineage and those that enhance the differentiated function of osteoblasts BMPs are found to be the only factors known to stimulate mesenchymal stem-cells to differentia-

te into an osteoblastic and chondroblastic lineage [12,57,63] Mitogens, such as bFGF and TGF-ß play a more important role in bone repair concerning cell proliferation, whereas at times of repair more cells are necessary [11]. The detrimental influence on the mesenchymal tissue ingrowth in the presence of high concentrations of mitogenic growth factors is probably due to imbalance in the growth factor cascade On the other hand, the experimental environment in the chamber, with a scarcity of pluripotent cells from marrow or periosteum and the absence of mechanical loading, also may have influenced the outcome. These properties of the RSBC make it suitable to study the effects of growth factors or bone graft processing on graft incorporation.

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CHAPTER

INCORPORATION OF MORSELLIZED BONE GRAFT UNDER CONTROLLED LOADING CONDITIONS. A new animal model in the goat.

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Journal of Orthopaedic Reseach (submitted)

ABSTRACT

The aim of this study was to assess the *in-vivo* effects of mechanical stimuli in the incorporation process of impacted morsellized bone grafts

For this purpose the Subcutaneous Pressure Implant (SPI) was developed for use in the goat This device can generate controlled loading conditions onto a fixed amount of bone graft in the distal femur Twenty goats were divided into three groups non-loaded, 2 MPa, or 4 MPa loads (1 Hz, 1 hour/day) The goats were killed after 3, 6 or 12 weeks The results were documented by clinical observations, quantitative bone density from QCT scanning and histomorphometry Nine post-mortem knee specimens were prepared in a similar manner to the experimental knees to determine the reproducibility and mechanical stability of the grafting method

Three goats were lost due to complications, the others functioned clinically well At 12 weeks, QCT bone density measurements revealed relevant differences between the three groups, demonstrating persistently high densities in the 4 MPa specimens, but reduced densities in the 2 MPa and non-loaded specimens Histologically, the initial repair process did not differ between the groups A front of vascular fibrous tissue with osteoclasts invaded and resorbed the dead bone graft, whereafter osteoblasts apposited new woven bone on the graft remnants However, at 12 weeks the loaded grafts had transformed into a vital trabecular structure, whereas non-loading had resulted in the disappearance of graft and trabeculae Morphometrically, the mineralizing surface was larger in the 4 MPa group ($p=0\ 02$), but the incorporation and remodelling processes had advanced more rapidly in the 2 MPa specimens ($p=0\ 04$)

Although no significant differences were observed in the process of early healing between the loaded and non-loaded specimens, loading was clearly an indispensable factor in the final transformation of bone allograft into a vital load-bearing trabecular structure

INTRODUCTION

Bone stock deficiency around failed arthroplasties can be reconstructed with bone grafts In the late seventies, we developed a revision technique for hip replacements using impacted morsellized bone graft combined with cement in the acetabulum [23] Later this technique was adopted for revisions on the femoral side [6] Both techniques demonstrated promising initial clinical results [7,24]

The histological evaluation of biopsies taken from acetabulae, previously reconstructed with the morsellized chip grafting technique, demonstrated graft incorporation with the formation of a new trabecular structure after 8 months [2] However, in retrieved specimens, the chip graft located in areas behind stress-shielded protrusio cups (Mueller cages) showed no signs of incorporation (unpublished result)

In an animal experiment to test our revision technique, we also found that prosthetic design factors affect the graft incorporation process [22] We hypothesize that these effects are related to the mechanical loading of the graft material during revitalization, incorporation and remodelling In addition, certain levels of mechanical stimuli probably have an optimal effect on the repair process

To study this hypothesis and to evaluate the most favourable loading values, we developed a model to simulate the load-dependent bone graft incorporation processes In this paper we describe the animal model and the results of a loading experiment on the goat using impacted morsellized bone allografts and a Subcutaneous Pressure Implant

MATERIALS AND METHODS

Implant

The Subcutaneous Pressure Implant (SPI) is composed of 3 elements ***(Fig. 1, 2, 3)***. Part A is made out of titanium and consists of a hollow screw (8x20 mm), opening into a cylindrical pressure chamber (50x20 mm). A stainless steel piston (B) with a 5 mm diameter and a driving plateau (diameter 25 mm) fits into part A. A stainless steel screw-cap (C) (diameter 38 mm) closes the implant. The screw-cap is connected to a subcutaneous air pressure cannula. Compressed air is conveyed through this cannula to the implant. The cannula is made of high-pressure-resistant poly-urethane and has a length of about 1.3 meters. A titanium cuff (2 cm length, moulded fibres, diameter 50 µm, porosity 80%, NV Beheart S.A. Zwevegem, Belgium) is fixed to the distal end of this cannula. The loading regime is regulated by intermediate control equipment, which is connected to the distal end of the cannula during the daily loading period ***(Fig. 4)***. Frequency (Hz) and air pressure can be adjusted with this equipment. In this way, the piston is force-controlled and creates its own displacement dependent on the flexibility of the graft.

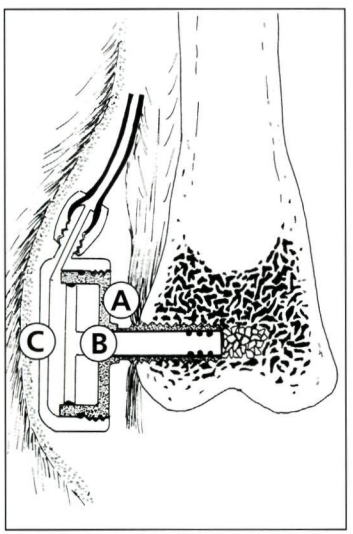


Figure 1
Subcutaneous Pressure Implant.
A = titanium screw
B = stainless steel piston
C = stainless steel screw-cap with air pressure cannula connector.

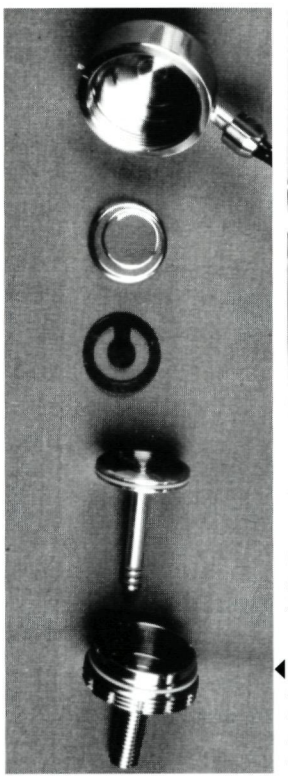


Figure 2
Photograph of seperate components of the titanium Subcutaneous Pressure Implant with polyurethane pressure cannula.

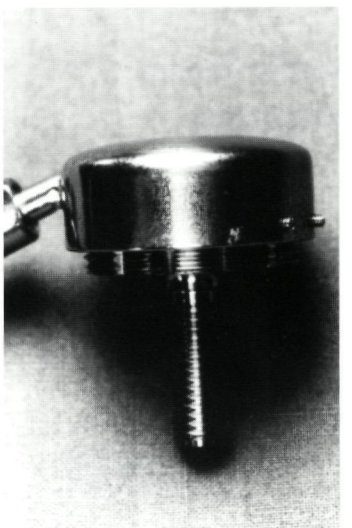


Figure 3
Photograph of the assembled Subcutaneous Pressure Implant

Surgical procedure

The goats (Capra Hircus Sana) were positioned on their side and the area of interest on the hind-leg was prepared according to a standard sterile surgical procedure. The knee was approached laterally, visualizing the collateral ligament. A hole, 30 mm deep, 6.7 mm diameter, was drilled at the femoral insertion of the lateral ligament, using a water-cooled diamond-tipped drill (Surgical Diamond Instruments®, Scientific Developments GMBH, Munich). The defect was filled with impacted morsellized bone

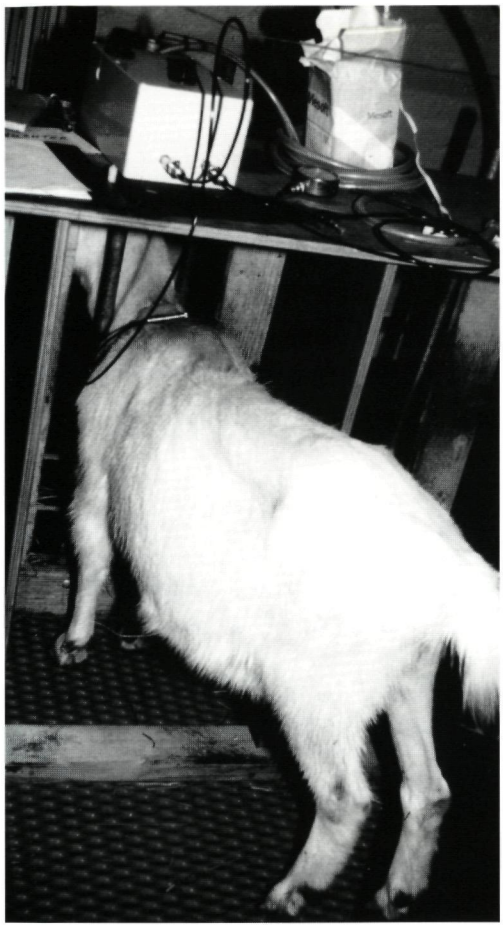


Figure 4
Experimental frame with a goat during the daily connection to the intermediate pressure control equipment.

graft over a distance of 10 millimetres, whereafter the implant was screwed into the pre-tapped hole. The amount of implanted morsellized graft was weighed. The air-pressure cannula was passed subcutaneously through a skin opening in the midline at the level of the first thoracic spine towards the implant on the lateral side of the knee. The cannula was fixed to the nipple on the cap of the implant, while the distal titanium-cuffed end of the cannula permanently extruded through the skin in the midline. This titanium cuff was placed subcutaneously near the skin opening to obtain fibrous tissue ingrowth and provide a stable canula-skin interface. After the implantation procedure, the animals received subcutaneous ampicillin (Albupen LA® 100 mgr/ml, Mycofarm) 7.5 ml per day for 5 days. During the remaining follow-up, no medication was administered to the animals. From the second week onwards, the implanted grafts were subjected to a daily loading regime.

Experimental design for ex-vivo study

To determine the reproducibility of the degree of graft impaction and the influence of the loading regime on the initial stability of the graft, nine post-mortem goat knees were prepared in a similar way as in the experimental animals. In six post-mortem specimens, the density of the graft was determined using QCT. The coefficient of variation for graft density due to repeated placement in the scanner was 4 per cent. Three post mortem specimens were subjected to loading, one knee with 2 MPa and two with 4 MPa. Radiographs were taken after 36000, 90000, 144000 and 198000 loading cycles, corresponding with 3, 6, 9 and 12 weeks of follow-up, respectively.

Experimental design for in-vivo study

Twenty goats received an SPI. Six goats served as controls with non-loaded pistons. Twelve goats were subjected to

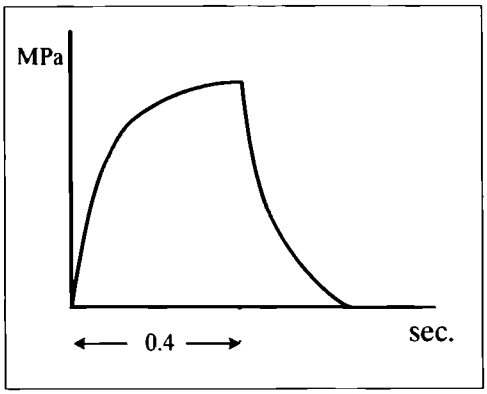


Figure 5
The ramp waveform pressure profile of the loading impulse The lag-time until maximal loading was 0 4 seconds

loading. The loading regimes were cyclic, 2 MPa (n=6) or 4 MPa (n=6), with a ramp waveform pressure profile ***(Fig. 5)*** and a frequency of 1 Hz, for 1 hour/day. The stress values chosen were in the range of physiological stresses around hip prosthesis components as calculated from finite element analyses [4 14 26]. The goats were grouped according to 3, 6 or 12 weeks of follow-up ***(Table 1)***. Three failures occurred during the experiment One goat in the non-loaded group developed an infection; there was air leakage in one goat in the 2 MPa group; one other goat in the non-loaded group developed aseptic loosening of the screw after 12 weeks. Two extra goats were operated on to compensate for two of these failures, so that each group comprised 6 goats, except for the 12-week-follow-up group which comprised 5 goats. All the goats received two doses of subcutaneous Calceine Green (20 mg/kg/day) for fluorescence microscopy seven days and one day before being killed.

Clinical evaluation, QCT and X-ray analysis

Clinical performance was graded according to Ypma et al.[27] (grade 0=leg not used at all, grade 1= supported incidentally, grade 2= loaded in standing position and incidentally while walking, grade 3= loaded in standing position and while walking but with a limp, grade 4= normal walking and standing pattern), while the skin reactions around the cannula were graded according to Holgers et al. [11] (grade 0= no irritation, grade 1= slight redness, grade 2= red and slightly moist tissue, no granuloma, grade 3= red and moist tissue and granuloma tissue, grade 4= infection).
Plain radiographs were taken in the A-P direction post-operatively and postmortem After excision the bone graft density was measured using QCT (Stratec XCT-960A).

Histological and histomorphometrical analysis

Specimens were fixed in 4% phosphate buffered formaldehyde. A cube of graft with surrounding bone was prepared, dehydrated in ethanol and embedded in PMMA. Undecalcified sections were made parallel to the long axis of the bone graft specimen (7μ) and processed for fluorescence microscopy or stained with Haematoxylin-Eosine or Goldner-Masson. Histomorphometry was performed using the PC-Image system (Foster Findlay UK), an automated computerized system or a semi-automated system with a microscope connected to a computerized video digitizing tablet system (Videoplan, Kontron Bildanalyse GMBH, Germany). For every variable in the analysis, two sections were used from the centre of the defect. The reproducibility of the morphometrical variables was expressed as the coefficient of variance (SD/$\bar{x}$) for repeated measurements. The Graft Volume, expressed as a percentage (GV = bone graft area/total tissue area of the defect; 25x, reproducability 2 5%), was measured with the PC-Image system The semi-automated interactive system was used to measure the Graft-Re-Vascularization percentage (GRV = area of revascularized graft/total graft area, 25x, reproducibility 5%), the amount of Graft Remnant expressed as a percentage (GRM = amount of dead graft in incorporated bone; 250x, reproducibility 12%), the Mineralizing Surface (MS =

double label surface + 1/2 single label surface/total bone perimeter, 100x, reproducibility 0.2%) and the Mineral Apposition Rate in µm/day (MAR = amount of bone apposition between a double label/6 days, 250x, reproducibility 4.4%)

Statistical analysis

The data were analysed statistically using 2-way ANOVA for the factors follow up and loading regime (2 Mpa, 4 Mpa and non-loaded) Results are presented as means with their standard deviations

RESULTS

Clinical results

Peroperatively, 0.8 ± 0.1 gram of impacted bone graft was implanted in the defects. The goats showed good function with no restraint at all (Ypma grade 4). All the implants and cannulae were encapsulated in fibrous tissue. The cannula skin passage showed only slight redness and some epithelial debris (Holgers grade 1). When tested after removal, all the implants were functioning smoothly and no body fluid had entered the inner chamber.

QCT and radiographic analysis

The average density of the trabecular bone surrounding the defect was 487.8 ± 36 mg/cm^3, while for the femoral cortical bone it was 1400 ± 84 mg/cm^3. In the *ex-vivo* specimens, the average density of the impacted graft was 664.7 ± 22.5 mg/cm^3. In the 3 week follow-up specimens, the densities did not differ significantly from the immediate postoperative values of 630.8 ± 121.6, 607.6 ± 152.7 and 678.3 ± 35.7 mg/cm^3 for the 2 MPa, 4 MPa loaded and the non-loaded grafts, respectively ***(Table 2)***. After 6 weeks of loading with 2 MPa or 4 MPa, the density had decreased to 546.0 ± 143.4 and 532 7 ±9.3 mg/cm^3 respectively, whereas a persistently high density of 637 3 ± 20.9 mg/cm^3 was observed in the non-loaded grafts. At 12 weeks, the density had decreased to 393 mg/cm^3 in the non-loaded graft. In the 2 MPa loaded grafts, it decreased to 343.9 ± 48 0 mg/cm^3, whereas the density in the 4 MPa loaded grafts remained high at 555.1 ± 37.4 mg/cm^3 As the densities did not change much during the first 6 weeks, the differences were not statistically significant (p=0.55). The 12-week folluw-up group was too small to conduct a separate statistical analysis.

The plain radiographs of the knees in the *ex-vivo* experiment did not demonstrate any piston movement into the graft, even after 198000 cycles of 4 MPa loading. In contrast, two pistons of the *in-vivo* loaded SPIs (2 MPa and 4 MPa) had started to protrude after 3 weeks, with a maximum of 2 mm at 12 weeks.

Histological and morphometric analysis

Histologically, only marginal graft revascularization had occurred at 3 weeks and there was no difference in histological appearance between the loaded and unloaded grafts A front of vascular mesenchymal tissue had advanced into the graft and osteoclasts had resorbed the dead bone graft. In the direct vicinity of the resorption activity, osteoblasts had apposited woven bone on the graft remnants, irrespective of the loading regime ***(Fig. 6A+B)***. At the graft-piston interface, a thin fibrous tissue layer was observed in three of the loaded specimens. In all the specimens loaded for 6 weeks, remodelling was seen in the host bone and adjacent graft, but incorporation and remodelling were more advanced in the 2 MPa loaded grafts, with large areas of woven bone apposited on dead trabeculae ***(Fig. 6C+D+E)***. At 12 weeks, the loaded grafts had transformed into a vital trabecular structure, which demonstrated load-dependent trabecular orientation and density, especially in the area on top of the piston ***(Fig. 6G)***. Non-loading, in contrast,

TABLE 1

Follow-up	non-loading (#)	2 MPa (#)	4MPa (#)	total number (#)
3 Weeks	2 (1a)	2 (1b)	2	6 (2)
6 Weeks	2	2	2	6
12 Weeks	1 (1c)	2	2	5 (1)
total number (#)	5 (2)	6 (1)	6	17 (3)

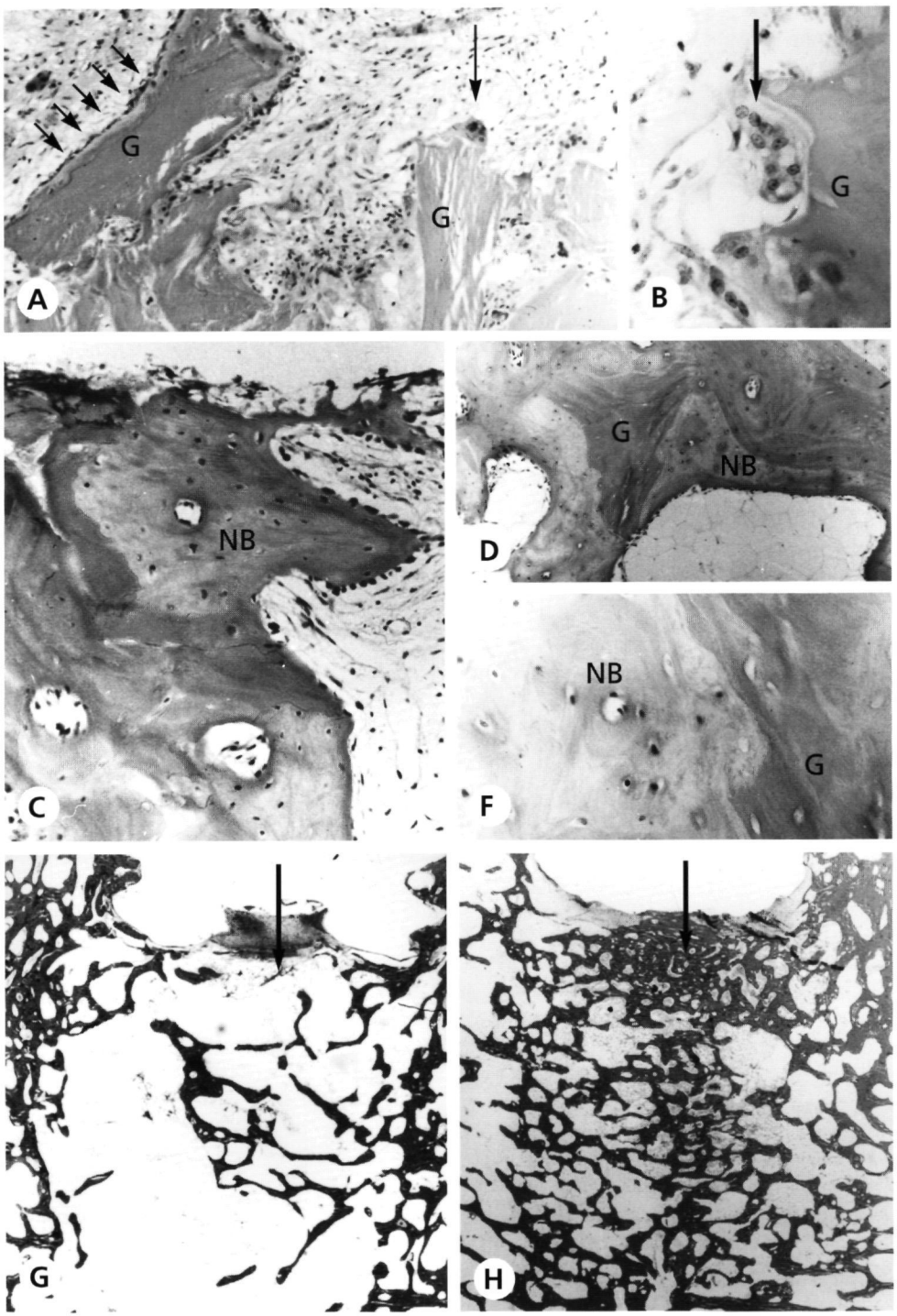
Table 1. *Experimental in-vivo study, numbers in brackets are failures, excluded from the analysis*
1a infection, 1b pneumatic complication, 1c aseptic loosening of the screw

TABLE 2

FU (wk)	F (MPa)	QCT (mgr/ccm)	GV (%)	GRV (%)	GRM (%)	MS (μm/day)	MAR
3	0	**678.3**	**56.5**	**22.7**	**78.5**	**0.43**	**3.0**
		35.7	*1.6*	*28.8*	*7.7*	*0.12*	*0.8*
3	2	**630.9**	**53.5**	**19.7**	**84.4**	**0.34**	**3.4**
		121.7	*2.5*	*27.8*	*6.8*	*0.12*	*0.5*
3	4	**607.6**	**52.3**	**10.0**	**86.5**	**0.40**	**3.5**
		152.7	*0.6*	*5.4*	*7.8*	*0 19*	*0.07*
6	0	**637.3**	**53.5**	**83.4**	**47.3**	**0.43**	**2.4**
		20.9	*9.5*	*13.1*	*2.5*	*0.01*	*0.6*
6	2	**546.0**	**43.1**	**87.2**	**38.3**	**0.45**	**2.7**
		143.4	*5.1*	*4.5*	*8.1*	*0.04*	*0.7*
6	4	**532.7**	**49.3**	**56.9**	**50.6**	**0.52**	**3.0**
		9.3	*11.5*	*37.7*	*12.4*	*0.01*	*1.1*
12	0	**269.0**	**17.8**	**100**	**17.8**	**0.43**	**2.8**
12	2	**344.0**	**28.4**	**100**	**4.7**	**0.45**	**1.6**
		48.0	*2.0*	*0*	*1.3*	*0.01*	*1.2*
12	4	**555.2**	**43.9**	**100**	**5.7**	**0.53**	**1.2**
		37.4	*4.3*	*0*	*1.4*	*0.06*	*0.4*

Table 2. *QCT and histomorphometrical results,* ***Mean****, SD*

Notes **GV** = bone graft area/total tissue area of the defect, **GRV** = area of revascularized graft/total graft area, **GRM** = amount of dead graft in incorporated bone, **MS** = double label surface + 1/2 single label surface/total bone perimeter, **MAR** = amount of bone apposition between a double label/6 days


G
G
A
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had resulted in the disappearance of the graft and trabeculae ***(Fig. 6F)***. Morphometrically, bone density followed the same pattern as in the QCT measurements and demonstrated lower densities in the non-loaded and 2 MPa loaded specimens after 12 weeks, whereas the density remained high in the 4 MPa specimens ***(Table 2)***. However, these differences were not statistically significant (P=0.2). The bone grafts had revascularized most rapidly in the non-loaded group and the 2 MPa loaded group, whereas revascularization in the 4 MPa group was slower until 6 weeks (P=0.09). There were significantly fewer dead graft remnants in the incorporated bone in the 2 MPa group than in the other specimens (P=0.04). The surface onto which woven bone had been apposited (MS) was significantly larger in the 4 MPa loaded specimens (P=0.02), but no statistically significant difference was observed in the speed of bone apposition (MAR) (P=0.9).

DISCUSSION

The Subcutaneous Pressure Implant enabled us to investigate the influence of controlled loading on the bone graft incorporation process *in-vivo* in the absence of any functional restriction or impairment of well-being of the animals. Mechanically induced bone repair has been described for cortical fracture models [1,10,18] and also recently for trabecular repair and adaptation [9,11]. Although the latter systems might also be used to study load-dependent bone graft incorporation, we decided to design an uncomplicated implant that operates on air pressure, is easy to apply, is fairly invulnerable to technical complications and involves less strain on the animals. The disadvantage of our experimental set-up was that only one type of graft was investigated per animal. Besides the study of morsellized bone grafts, our model can also be used to study the influence of loading on the incorporation of processed grafts or any other biomaterial.

When starting our project, we initially designed a percutaneous implant similar to the subcutaneous one. With this percutaneous implant, which permanently pierced the skin in the lateral knee region of the goat, we hoped to prevent and control potential pneumatic complications, such as air leakage and body fluid penetration into the implant. Several percutaneous implants were applied successfully [16], but a stable implant-skin interface could not be created [25], due to tissue movement at the knee, which resulted in infection and loosening of the implant. Our specially designed second implant as described in this paper, did not display these clinical problems.

In designing the experimental set-up, statistical calculations were made using pilot experimental results to determine the number of animals necessary. However, the six animals per group demonstrated larger interanimal variations than had been expected on the basis of the pilot experiment. This is reflected clearly by the inconsistent standard deviations in the QCT results. In contrast, after 3 weeks of follow-up, no differences of any kind could be demonstrated between the 2 MPa and 4 MPa loaded groups. However, the remaining number of animals in the 12 weeks follow up group was too small to conduct a separate statistical analysis. Although the statistical calculations did not substructure the histological observations concerning all parameters, loading had

Figure 6

A+B A 3 week specimen with fibrovascular tissue advancing into the graft Osteoclasts resorb the dead bone graft, single arrows (G) In the direct vicinity of the resorption activity, osteoblasts apposit woven bone on the graft remnants, four arrows (G) [A HE, 100x, B HE, 250x]

C+D+E Incorporation of the dead bone graft and extensive remodelling activity in a 6 weeks loaded specimen A mixture of dead graft (G) and immature woven bone (NB) is present [C HE, 100x, D HE, 65x, E HE, 250x]

F A 12-week non-loaded specimen The graft material is dissapeared and is not replaced by new vital trabeculae [HE, 12x]

G A 12-week 4 Mpa loaded specimen The loaded grafts had transformed into a vital trabecular structure, which demonstrated load-dependent trabecular orientation and density, especially in the area on top of the piston (arrow) [HE, 12x]

definitely influenced the incorporation and remodelling of the bone grafts after 12 weeks. Longer-term follow-up studies are needed to confirm these observations.

Plain radiographs and QCT of the cadaver specimens demonstrated a reproducible experimental graft-impaction technique. The fact that the piston remained stationary after extensive *in-vitro* loading, demonstrated initial mechanical stability of the graft. Radiographic evidence of migration of the piston after 6 weeks of loading, combined with histological evidence of graft incorporation and remodelling, can be explained by a temporary reduction in mechanical rigidity due to the simultaneous occurrence of graft resorption and bone apposition. This local remodelling process, together with the postoperative observation of permanent deformation of the impacted bone chips, can to a certain extent explain the subsidence of the femoral component in clinical studies [5 19].

The incorporation behaviour of the morsellized bone graft used in our experiment is in accordance with histological findings in other animal experiments [20 21 22]. The same pattern of revascularization was described, with an invasion front of loose connective tissue, local osteoclastic bone destruction combined with bone formation on the remnants of the graft. In our model, however, this process advanced more rapidly In the studies by Schreurs et al.[21] and Schimmel et al.[20], the graft layer was fully revascularized after 12 weeks, whereas in our experiment, this had already occurred after 6 weeks. Two explanations can be given for this phenomenon. First, in the acetabulum and femur the vessels can only penetrate into the graft layer from one side, whereas in our model, this could occur from all sides. Second, the amount of vascular damage in reamed host bone is more extensive than in our water-cooled diamond-drilled defect.

Our experiments showed that in the first 6 weeks of follow-up, the bone graft repair reaction was relatively independent of the loading regime. Apart from a detrimental influence on revascularization in the high-load specimens, no differences were observed in bone density and the bone mineral apposition rate between the three groups. These similarities between the groups at 3 and 6 weeks and the small numbers that do not allow statistical calculations on separate groups, gave rise to a lack of statistically significant results. It seems that at the very beginning of repair after surgical trauma, there is an all-or-nothing reaction probably caused by various cytokines and pluripotent mesenchymal cells [3 13]. At longer-term follow-up, the size of the bone mineralizing surface and bone density were directly correlated with the amount of load applied. This suggests that subsequent loading influences the later stages of graft incorporation and remodelling. The final trabecular orientation in the loaded specimens indicates that loading plays an important role in transforming the incorporated bone allograft into a new vital bone structure that is able to withstand load and adapt to the local mechanical environment. To determine the actual receptors and effectors in the bone graft remodelling process and to distinguish more clearly between initial trauma and loading effect, longer-term experiments are needed.

The sequence of events that occurred in our specimens was similar to that in the incorporation process of impacted morsellized bone graft in the human acetabulum [2] and femur [15 17]. The rate of incorporation, however, is much slower in the human than in the goat. Revascularization of the graft in patients takes at least four months, while the conversion of the dead graft into a vital trabecular structure with various amounts of graft remnants takes about eight months [2]. On the basis of our observation of decreased vascularization under high loading, it is tempting to advice against unrestricted weight bearing in patients who have received an impacted morsellized bone allograft. To further clarify the role of loading in the clinical behaviour of an impacted morsellized bone graft, Finite Element studies are required that use mechanical data from *in-vitro* studies [8], experimental animal studies, and human retrieval studies

To our knowledge no studies have been performed in which the incorporation patterns of morsellized fresh frozen bone allograft have been investigated under controlled mechanical conditions. The influence of mechanical loading on the fracture healing process has been investigated using segmental defect models in sheep [1,10]. The cytokines present in the cortical callus tissue will play the same role in this case as in the healing of bone grafts. In fractures stabilized with external fixation, that generate controlled mechanical loading, there were no obvious histological differences in healing until 10-12 weeks of loading. Underloading resulted in immature callus tissue [10], whereas subsequent increased physiological loading and cyclic loading resulted in enhanced bone formation [1,10]. From these and our experiments it appears that in the early response to trauma, a consistent cellular repair reaction occurs that is unrelated to loading, whereas the final healing and remodelling of bone tissue is dependent on loading.

In conclusion, our new animal model, using the Subcutaneous Pressure Implant, is suitable to study bone graft incorporation under controlled experimental loading conditions. Our experiment demonstrated a detrimental influence of high loading on the revascularization of impacted morsellized bone allograft and a more optimal rate of repair and remodelling with medium load. However, loading has been found to be an indispensable factor in turning dead morsellized graft into a vital trabecular structure. Further animal experiments with Finite Element Analysis of the impacted morsellized allograft reconstructions are necessary to map the precise effects of loading on this type of bone graft combined with total joint replacements.

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CHAPTER
DISCUSSION

DISCUSSION

Total joint replacements are one of the most succesfull interventions in treating disabled patients due to osteoarthritis However, all prosthesis do have a limited life-time The bone loss, generated during the mechanical loosening process, can be restored by using bone grafts The use of bone grafts in revision surgery of the joints will expand steadily in the near future This increase is a direct result of an absolute raise in the number of elderly people in the population and of broadening the indication for arthroplasties in the younger patient Because the revision operation is the 'second line of defence', it is important that the revision with bone grafts is conducted as optimal as possible Various revision techniques have been developed in the past, however these do not provide for a long term solution Although the clinical results of the morsellized bone grafting technique at the acetabular and femoral side are very promising, it is our clinical, experimental and moral responsibility to bring this techique further to perfection and discover potential pitfalls Bone grafts do have a large field of application, and the presented results comprise a broader field than the exclusive orthopedic utilisation of bone grafts

The enormous variation in type and size of bone grafts and its diversity in effectivity, demands controlled experimental research to unravel the influence of the various factors involved in failure or success The title of this thesis implicates a binary classification of influences on graft incorporation in terms of biological and mechanical This is an artificial separation In fact all processes in living bone are biological and although loading is a physical quantity, its expressions are executed by biological entities, like bone cells and their cellular transmittors [5 8] For the clinical pratice it is of importance to investigate those representants of the mechanical and biological categories that are applicable or recognisable in the clinical situation

First the potential role of the host bone bed in the failure or success of bone graft incorporation was investigated Because no data were available concerning the quality of host bone bed, biopsies were taken both at primary and revision surgery Biopsy material has its limitation, first the amount of material available for evaluation is limited and displays a large variability of bone structure over small distances [6 19] This variability was also found in our acetabular biopsies Although material could be taken from three areas in the acetabulum, the high standard deviations allowed no statistically significant differences, but a clinically relevant trend of sufficient vascularity in periprosthetic bone was demonstrated Alhough vascularity seems not to be confined in the revision bone bed, the observation of local wear particle material between the trabeculae in the revision specimens suggests another possible failure scenario of lack of bone graft incorporation Studies on interface tissue of mechanically failed implants revealed that activated macrophages, which had phagocytosed wear particles, stimulate the osteoclasts to resorb bone, leading to a condition of progressive bone loss [9 16] The presence of the wear particles in our revision biopsies could give rise to localized bone graft resorption in the future if not thoroughly removed during the revision operation The ultimate influence of the host bone on the success or failure of a bone graft reconstruction can only be determined in a prospective study, from all patients that recieved a bone graft, it is recommended to take biopsies from standardized areas of the graft-host bone contact

When chosing to investigate experimentally biological and mechanical factors, it became clear that two animal models had to be developed in order to define controlled loaded and non-loaded conditions When designing an animal model, one should contemplate about the choice of the animal, amount of animals to put on related to the inter animal variation, and the feasibility of the technical demands Both models have been designed for use in the goat One of the reasons for chosing the goat

as an experimental animal was because bone dimensions appeared sufficient large for the loading model Secondly, the goat is a strong animal that recovers quickly after surgery Thirdly, when taking the cost-benefit into account, the goat is an acceptable experimental animal for comparison with the human situation and last but not least our laboratory built up a large expertise with this type of animal For both models statisti cal calculations had been made using the pilot experimental results to determine the size of the groups and the lowest statistically acceptable number of animals was operated on The group of the bone chamber experiment was sufficient large for statistical analysis, but the animals of the loading experiment did demonstrate a larger interani mal variation than had been expected from the pilot experiment Therefore the statistical calculations of the loading experiment are not a clear reflection of the histological observations Series with larger animal numbers are therefore neccessary

To investigate the pure biological influences of implanted material, interfering loading influences have to be elimitated In order to fulfil this prerequisite, a bone chamber model, developed for use in rodents [12] was restyled and adapted for use with repeated harvest in the goat The advantage of this model is an intra-animal control and repeated harvesting in the same animal, which favours the discriminating power of the model and reduces the number of expensive and large laboratory animals that have to be sacrificed For the clinical practice this model can be of value, in discriminating quickly the advantage of new biomaterials or modulation factors, and justify to rate new trends at its potential value

Growth factors involve a broad field of chemical substances that display an enormous palet of effects on growing and healing tissue In the field of reconstructive surgery the regeneration potential of these growth factors could compensate for insufficient intrinsic healing potency From the enormous number of factors, TGF-ß, rh-BMP-2 and b-FGF have been chosen to investigate for its value in bone graft incorporation, because they are amongst the factors that are relatively easy to obtain and have proven effective in some experimental studies The mitogens TGF-ß and b-FGF could not stimulate bone ingrowth, but had an inhibitory influence perhaps due to an imbalance in the growth factor cascade BMP-2 is known to induce differentiation and was in the bone chamber experiment able to increase the bone ingrowth at specific concentration These observations underline the difficulties for clinical applications of growth factors The concentration and application-form determine directly the influence on the healing process Although BMP-2 seems a potential factor in clinical cases with inferior healing capacities and absence of loading, the application of growth factors in the bone grafting surgery has to be dissuaded until a reproducible delivery system with a consistent and controllable positive outcome is available

Although an influence of loading in the bone graft incorporation process was suspected, its role had never been objectivated From experiments on fracture healing and trabecular repair and adaptation, mechanical loading appeared to have a substantial role [4 11 12 15] The simulation models in the goat of this biological reconstruction method [17 18] were not suitable to standardize and study the effect of the local loading situation in the graft layer A prerequisite in designing the new model, was that the implant had to be uncomplicated, easy to apply, fairly invulnerable to technical complications and provoke less strain on the goat Therefore we first developed a pneumatic driven implant that was used percutaneously in the knee in order to prevent and control potential pneumatic complications The prototype generated infections and failure, however, an adaptation to a subcutaneous one could be applied successfully

During the loading experiment, the morsellized bone allograft was put under compressive load that was in the range of physiological stress patterns around hip pros-

thesis as known from finite element analyses [7,13,21]. The dichotomy in two loading regimes is an artificial one. Although the loaded specimens displayed an incorporated graft and remodelled trabecular bone, it has to be realised that the chosen loading regiments possibly are not the most optimal ones. Similar to the concentration-specific effects in the growth factor experiment, it can be hypothesized that an optimal combination of load magnitude, frequency and duration is able to stimulate the graft incorporation. In other words, a window of optimal mechanical influences can be operational in the graft incorporation process, whereas stimulatory settings outside this window display no, inadequate or a detrimental effect. These speculations need further experimental research.

When comparing the experimental model to the clinical situation of impacted morsellized graft at the acetabular and femoral side, a few critical notes have to be made. In the acetabulum and femur the blood vessels can penetetrate the graft layer only from one side, whereas the loaded specimens are embedded within a circumferential vascular surrounding of healthy trabeculae and marrow. This explains to some extent the fast experimental graft incorporation compared to the human situation. The species specific rate of metabolism plays of course also a major role. Secondly, the specimens are loaded in compression, whereas in the clinical situation shear and tension stresses are also present in the graft-cement-bone combination. Is it not possible that these tension and shear stresses are sensed by the same bone cells during the incorporation process, equal to the response to compressive load? In-vitro studies revealed mechanical excitation of bone cells through bone fluid shear stresses, irrespective of the axis and direction of loading [22] Cement has not been a research item for this thesis, however, it has to be noted that the graft-cement-bone combination in the reconstruction method serves as a indissolubly connected entity. The clinical relevant question rises whether the cement layer could be more vulnerable to failure in due time to tension and shear than the bone graft layer with its adaptive capabilities. Despite the confined loading-axis and direction in our mechanical model, it can be of great value to determine in the future the behavior of graft-cement implants under compressive load, and also to assess the applicability and advantages of bone graft substitutes like hydroxyapatite, tricalciumphosphate and growth-factor enriched bone grafts under loaded conditions.

The mobilisation scheme of patients that received a morsellized bone graft has undergone dramatic adaptations in time. In the early days, patients were not allowed to partially load their hip until 6 weeks after the operation, whereas nowadays patients are encouraged to get out of bed within one week after the operation. These changes were not only introduced to lower the risk of trombosis, but also because the thought existed that bone had to be loaded in order to heal. These opinions now can be substructured by our experimental observation that mechanical excitation of the graft-material at longer term follow-up resulted in the formation of a vital trabecular structure. However, on the basis of our observation of decreased vascularization under high loading, it is tempting to advise 'start slow, go low' in the unrestricted weight bearing

As already stated in the introduction, one of the initiations of this research was the question concerning the difference in incorporation and clinical results between morsellized and structural allografts. From the bone chamber experiment, no clinically important difference in bone growth stimulatory activity from the fresh frozen morsellized or structural cancellous allograft could be detected. Therefore, a small pilot experiment was conducted using structural cancellous bone plugs under defined loading conditions. All the cancellous grafts had dissapeared after 6 weeks, either loaded or not, whereas the morsellized grafts incorporated into a vital trabecular graft. We have tried

to obtain explanations for these phenomena, but despite the experiments we still are not able to unravel the differences between the morsellized bone graft and the structural graft. A hypothetical explanation for the resorption of the structural graft could be that the amount of bone in the cancellous bone plug was insufficient to act as a trellis for woven bone apposition because of a massive resorption reaction during the primary surgical repair reaction. Another reason could be that the necrotic marrow tissue present in these bone plugs generated a florid immunological reaction with subsequent bone resorption. Also the inadequate transmission of loading in the bone plugs could be responsible for the lack of graft incorporation and new bone formation. To further unravel the role of loading and the influence on the morphology of the bone graft in the bone graft incorporation process, additional experiments are being performed.

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SUMMARY

Total hip replacement is a successful intervention in orthopaedic surgery. In time the arthroplasty can fail due to aseptic loosening, causing periprosthetic bone loss. The loss of bone stock around arthroplasties is a multifactorial process. Factors involved in this process are discussed in **Chapter 2** and include resorption due to a foreign body wear particle reaction, patient specific influences, immunological events and mechanical instability of the implant.

In the past various surgical revision techniques have been developed to compensate for bone loss and to restore the stability of the new implant. The best long-term solution was the use of bone grafts to restore the deficient peri-implant bone. From clinical observations it became clear that the size and structure of a bone graft determined to a large extent the clinical success and failure of a revision reconstruction with bone graft. Compact structural bone grafts appeared only a short time solution; this type of bone graft can fail due to incomplete incorporation, resorption and collapse of the necrotic central part. At our Department of Orthopaedics a revision technique, using impacted morsellized cancellous bone grafts, hase been developed to restore acetabular and femoral defects. Clinically this technique is successful. In order to map the processes involved in the incorporation of this type of graft and to bring the use of this technique further to perfection, it is necessary to enlarge the basic knowledge. Therefore factors involved in the incorporation process of morsellized bone graft were studied in human material and experimental models.

The bone graft incorporation process is dependent on host bone and graft specific properties. During the incorporation process the graft consolidates to the host bone. A prerequisite for this to occur is a sufficient vascularity and transport of mesenchymal cells from the host bone to the graft.

An often stated opinion is that failure of an arthroplasty leads to sclerotic and avascular periprosthetic bone and therefore potentially jeopardizes the graft incorporation. To objectify this opinion, the viability and vascularity of the acetabular bone bed has been compared in biopsies taken at primary and revision total hip arthroplasty, which is described in **Chapter 3**. It appeared that the acetabular bone bed at revision surgery, with its specific morphological appearance, was viable, with sufficient vascular supply and remodelling activity to form an acceptable basis for bone grafting combined with revision arthroplasty.

From simulation animal experiments it was clear that both in the acetabulum and the femur the impacted morsellized bone graft incorporated completely and remodelled into a vital new trabecular structure. To determine the speed and completeness of the incorporation in the clinical situation, biopsies were taken from grafted acetabulae in patients. **Chapter 4** describes the graft incorporation in human. Compared to the animal experiments, the sequence of events was qualitatively equal, however, the timely interval in human is much slower. The biopsies indicated that 8 months post-revision, the graft had incorporated into a new trabecular structure, initially with woven bone that subsequently remodelled into lamellar bone.

When investigating experimentally the role of factors in the incorporation process, it is of importance to standardize the experimental conditions. Two animal models were developed to study the role of biological factors (graft dependent qualities, growth factors dependent incorporation) and mechanical factors (load dependent incorporation). In **Chapter 5** the 'repeated sampling bone chamber' is described that allows studying bone and tissue ingrowth into bone grafts under non-loaded conditions. The chamber is made of titanium and is implanted in the tibia of the goat. The implant is designed for repeated harvest, so that the inter-sample variability and num-

ber of experimental animals to be used is reduced The chamber is easily placed and well tolerated for long term implantation The inherent low bone and tissue ingrowth into the chamber, make this implant suitable to study bone inductive substances

The influence of bone inductive substances in stimulating the bone healing and graft incorporation under non-loaded conditions is described in **Chapter 6.** A large number of growth factors is known for its bone inductive activity, however TGF-ß, rhBMP-2 and b-FGF have been investigated because they are amongst the factors that are relatively easy to obtain and have proven effective in some experimental studies The mitogens TGF-ß and b-FGF could not stimulate bone ingrowth, but had an inhibitory influence perhaps due to an imbalance in the growth factor cascade rhBMP-2 was able to increase the bone ingrowth at specific concentration and therefore is potentially the most suitable alternative in cases that require additional bone induction However, a reproducible outcome in the utilisation of growth factors is yet not obtainable and additional research therefore has to be performed before a general clinical application is performed

Studies on fracture healing revealed that the bone healing process was under influence of mechanical loading An influence of mechanical loading conditions on the graft incorporation and remodelling process was suspected from human biopsy material taken from acetabular morsellized bone graft reconstructions and animal experiments testing our revision technique To verify this observation and to evaluate favourable loading values, an animal model was developed which is described in **Chapter 7** A device was designed and manufactured that generates controlled loading conditions on a fixed amount of bone graft in the distal femur of a goat No significant differences could be observed in the process of early healing between the loaded and non-loaded specimens, however, the group with the highest loading demonstrated a retarded revascularization At longer term follow-up, non-loading resulted in resorption of the graft, whereas loading was an indispensable factor in the final transformation of bone graft into a vital trabecular structure with a load dependent density It appears that in the early response to trauma a massive cellular reactions is generated independent of loading, whereas the final healing and remodelling of bone graft depends on local loading conditions

In this thesis we have objectified biological and mechanical factors involved in the incorporation process of impacted morsellized bone grafts Questions still remain concerning the difference in incorporation potency of structural and morsellized bone graft However, animal models now are at our disposal in which we can enlarge our knowledge and clarify further the role of certain cell populations, growth factors and windows of mechanical loading involved in the healing and incorporation of bone graft

SAMENVATTING

De totale heup vervanging is een succesvolle ingreep in de orthopedische chirurgie. In de loop van de tijd kan een gewrichtsvervangende prothese gaan falen ten gevolge van aseptische loslating en botverlies veroorzaken in het omgevende botbed. Het verlies van bot rond een prothese is het gevolg van een multifactorieel proces. De betrokken factoren zijn beschreven en bediscussieerd in **Hoofdstuk 2**, en betreffen onder andere een vreemdlichaamreactie tegen slijtage partikels, patient specifieke invloeden, immunologische gebeurtenissen en mechanische instabiliteit van het implantaat.

In het verleden zijn verschillende chirurgische technieken ontwikkeld als oplossing voor het probleem van het botverlies en het herstellen van de stabiliteit van het nieuwe implantaat. Het gebruik van botgrafts bleek de beste oplossing om de deficiënte botmassa rond het implantaat te herstellen voor lange termijn. Vanuit de kliniek werd duidelijk dat de grootte en vorm van de botgraft uiteindelijk het klinische resultaat bepaalde van de bot graft-revisiereconstructie. De compacte structurele botgrafts bleken alleen te voldoen als korte termijn oplossing; dit type graft kan falen ten gevolge van incomplete incorporatie, resorptie en collaberen van de necrotische kern van de graft. De afdeling Orthopedie van het Academisch Ziekenhuis Nijmegen heeft een revisie-techniek ontwikkeld waar gebruik wordt gemaakt van geimpacteerde trabeculaire botfragmenten en cement om defecten in het acetabulum en femur te herstellen. Deze techniek is klinisch succesvol. Om het gebruik van de fragment botgraft verder te perfectioneren en de processen die tijdens de incorporatie van de botgraft een rol spelen beter te begrijpen en te beschrijven, is het noodzakelijk de basiskennis te vergroten. Daarom zijn de factoren die een rol spelen in het incorporatieproces van geimpacteerde fragmentgraft bestudeerd in humaan materiaal en experimentele modellen.

Het botgraft-incorporatieproces staat onder invloed van het omgevende gastheerbot en botgraft specifieke eigenschappen. Tijdens het incorporatieproces consolideert de graft met het omgevende gastheerbot. Een voldoende doorbloeding en transport van mesenchymale cellen vanuit het omgevende gastheerbot naar de botgraft zijn een voorwaarde voor een goede consolidatie en incorporatie.

Er wordt vaak beweerd dat het falen van een prothese aanleiding geeft tot het vormen van sclerotisch en avasculair gastheerbot, waardoor een goede graft incorporatie potentieel in gevaar wordt gebracht. Om deze opvatting te kunnen objectiveren zijn er biopsieën genomen van het acetabulaire botbed tijdens primaire en revisie operaties en zijn de groepen vergeleken met betrekking tot hun vitaliteit en vascularisatie. De bevindingen zijn beschreven in **Hoofdstuk 3**. Het is gebleken dat het acetabulaire botbed tijdens revisie een specifiek morfologisch beeld vertoond van vitaal, goed gevasculariseerd bot met remodelerings-activiteit; het vormt een acceptabele basis voor een botgraft revisie reconstructie.

Dierexperimenten waarin de revisie techniek is nagebootst laten zien dat zowel in het acetabulum als femur de geimpacteerde fragmentgraft volledig incorporeert en remodelleert tot een nieuwe, vitale trabeculaire structuur. Biopsieën zijn genomen van acetabulaire grafts in patienten om de volledigheid en snelheid te bepalen van de graftincorporatie in de klinische situatie. In **Hoofdstuk 4** wordt het graft-incorporatieproces zoals dit in de patiënten plaatsvindt beschreven. Vergeleken met de dierexperimenten is de volgorde van gebeurtenissen vergelijkbaar, maar is de snelheid van het proces veel langzamer in het patiënten-materiaal. De biopsieen geven aan dat 8 maanden na de revisie de graft is geïncorporeerd in een nieuwe trabeculaire structuur, die initieel veel jong bot bevat en vervolgens remodelleert tot lamellair bot.

Bij het bepalen van de rol van specifieke factoren in het incorporatieproces van botgraft, is het belangrijk dat de experimentele condities gestandariseerd worden Twee diermodellen zijn ontwikkeld om de rol die biologische (graft specifieke kwaliteiten, groeifactor afhankelijke incorporatie) en mechanische factoren (belastingafhankelijke incorporatie) spelen, te bepalen In **Hoofdstuk 5** wordt de 'repeated sampling bone chamber' beschreven Dit implantaat maakt een onderzoek mogelijk naar de bot- en weefselingroei in botgrafts onder niet belaste condities De botkamer is vervaardigd uit titanium en wordt geimplanteerd in de tibia van de geit De botkamer is ontworpen voor herhaald oogsten' zodat de onderlinge variabiliteit in specimen en het aantal te gebruiken experimentele dieren verminderd wordt De botkamer kan gemakkelijk geimplanteerd worden en wordt voor langdurige implantatie getolereerd De inherente lage bot- en weefselingroei maken dit implantaat geschikt voor het bestuderen van botinductieve substanties

De invloed van botinductieve substanties op de genezing en incorporatie van botgraft onder niet belaste omstandigheden is beschreven in **Hoofdstuk 6** Een groot aantal groeifactoren is bekend om zijn botinductieve activiteiten, maar TGF-ß, rhBMP-2 en b-FGF zijn onderzocht, omdat deze factoren relatief gemakkelijk te verkrijgen zijn en ook effectief bevonden zijn in experimentele studies De mitogenen TGF-ß and b-FGF konden de botingroei niet stimuleren, maar hadden daarentegen zelfs een remmende invloed, mogelijk ten gevolge van een disbalans in de groeifaktorcascade rhBMP-2 was in een bepaalde concentratie in staat de botingroei te vermeerderen en is daarom potentieel het meest geschikte alternatief om in specifieke gevallen botinductie te veroorzaken Maar een reproduceerbare uitkomst in het gebruik van groeifaktoren is tot op heden nog niet mogelijk Aanvullend onderzoek is noodzakelijk om de effectiviteit verder te objectiveren en te waarborgen alvorens een algemene klinische toepassing wordt doorgevoerd

Studies met betrekking tot fractuurgenezing geven aan dat het botgenezingsproces onder invloed staat van mechanische belasting In humaan biopsiemateriaal van acetabulaire fragmentgraft reconstructies en in materiaal van dierexperimenten waarin de revisietechniek nagebootst werd, werd duidelijk dat de graft incorporatie ook mogelijk onder invloed stond van mechanische belastingsomstandigheden Om deze observaties te verifieren en om potentiele optimale belasting parameters vast te stellen, werd een diermodel ontwikkeld dat beschreven staat in **Hoofdstuk 7** Er werd een implantaat ontworpen en vervaardigd dat gecontroleerde belastingen kon produceren op een vaste hoeveelheid geimpacteerde botfragmenten die geimplanteerd werden in het distale femur van de geit Er konden geen significante verschillen worden gevonden in het vroege genezingsproces tussen specimens die belast waren of niet belast waren Alleen de groep met de hoogste belasting liet een vertraagde revascularisatie zien Na langere follow-up resulteerde een afwezige belasting in het resorberen van de graft, terwijl na belasting de botgraft was getransformeerd in een vitale trabeculaire structuur met een belasting-afhankelijke dichtheid Het lijkt erop dat tijdens de vroege response ten gevolge van het operatietrauma er een massieve celreactie ontstaat die onafhankelijk is van de belasting, terwijl in de uiteindelijke genezing en remodellering van botgraft de lokale belasting een belangrijke rol speelt

In dit proefschrift hebben we biologische en mechanische factoren geobjectiveerd die een rol spelen in het incorporatie proces van botgrafts Er blijven nog steeds vragen onbeantwoord ten aanzien van het verschil in incorporatie-gedrag en mogelijkheden van structurele en gefragmenteerde graft Maar momenteel staan ons diermodellen ter beschikking om onze kennis te vergroten en vragen op te lossen ten aanzien van de rol van specifieke celpopulaties, groeifaktoren en windows' van mechanische belasting in het genezings- en incorporatiegedrag van de gefragmenteerde botgraft

DANKWOORD

Toen ik in 1994 aan het experimentele onderzoek rond de 'morsellized bone graft' begon kon ik mij natuurlijk geen voorstelling maken van de fases van inspiratie, motivatie, transpiratie en 'desperatie' die elke onderzoeker moet doormaken Natuurlijk begon ik met volle overtuiging aan dit uitdagende project om dit 'geitje' wel eens eventjes te wassen
Maar de 'wet van Murphy' was ook in mijn onderzoek van toepassing Onderstaande personen ben ik veel dank verschuldigd, omdat het dankzij hun inbreng, stuwende kracht en motiverende woorden het steeds weer mogelijk was de draad op te pakken en de noeste arbeid om te toveren tot dit proefschrift

Prof Dr T J J H Slooff (Orthopedie KUN) mentor van de morsellized revisie-techniek, promotor en steun en toeverlaat

Prof Dr R Huiskes (Orthopedie, Biomechanica KUN) begeleider, bron van ideeen en kritische noten en promotor

Dr P Buma (Orthopedie, Histomorphologie KUN) dagelijks begeleider en adviseur, rustpunt en probleemoplosser tijdens 'onverwachte' situaties en co-promotor

Dr J W M Gardeniers (Orthopedie KUN) adviseur vanuit klinisch oogpunt en hulp bij het verzamelen van humane biopten

Dr B W Schreurs (Orthopedie KUN) kritisch referent en stuwende kracht in het continueren van de klinisch-experimentele en dierexperimentele vervolgstudies rond de morsellized botgraft problematiek

W van de Wijdeven (Orthopedie, Biomechanica KUN) technisch adviseur bij het ontwikkelen van het belasting-implantaat en hulp bij het uitvoeren van in-vitro experimenten

Mw H Versleyen (Orthopedie, Histomorphologie KUN) hulp bij het vervaardigen van enorme hoeveelheden histologische (kwaliteit) coupes en het oplossen van praktische problemen

Dr P Aspenberg (Orthopedics, University Lund, Lund, Zweden) adviseur bij de botkamerexperimenten en gewaardeerd sparring partner in discussies rond de botgraft-problematiek Dank voor het ter beschikking stellen van b-FGF

Dr R de Graaf (Medische Statistische Afdeling KUN) hulp bij de statistische fundering van de dierexperimenten en uitvoerder van de statistische bewerkingen van de experimentele data

A Peters, P Philipsen, Th Arts (Centraal Dieren Laboratorium KUN) deskundige assistenten bij het opereren van de proefdieren en praktische 'meedenkers'

Prof Dr W van de Berg (Rheumatologie KUN) het ter beschikking stellen van TGF-ß

Prof Dr J A Jansen (Tandheelkunde, Biomaterialen KUN) het ter beschikking stellen van titanium meshes, waardoor lange termijn belastingexperimenten mogelijk werden

Dr A Ederveen (Organon Nederland) het ter beschikking stellen van de Quantitatieve CT

Prof Dr K Draenert (Zentrum fur Orthopaedische Wissenschaften, Munchen, BRD) adviseur bij de technische/praktische aspekten van de dierexperimentele modellen

Reza Sardar, Peter Keijzer, Tony van Tienen, Timothy Radstake, Harm-Willem Boons en Niels Veltman (Studenten Geneeskunde) dank voor de inzet en de gezelligheid tijdens het uitvoeren van de dierexperimenten en de histologische metingen

Tijdens gezellige theepauzes werd vaak in een ongedwongen sfeer menige onderzoeks-, politieke of morele noot gekraakt en kon 'the mind' weer even worden 'ge-reset'
Dr Kuipers, Reinout, Theo, Els en Edith, bedankt!

Mijn lieve ouders wil ik graag bedanken voor hun nimmer aflatende zorg en inspirerende levenswijsheid

Als laatste genoemd maar doorslaggevend voor het volbrengen van de 'Bone graft Mission' Lieve Henk, jouw onvoorwaardelijke steun, peptalks en verwennerijen waren (en zijn nog steeds) voor mij van ongekende waarde!

CURRICULUM VITAE

Nancy Maria Petra Lamerigts werd geboren 5 maart 1968 te Beegden (L) Ze behaalde haar VWO diploma aan de scholengemeenschap St Ursula te Horn in 1986 en behoorde tot de gelukkigen die meteen werden ingeloot voor de studie Geneeskunde In 1990 behaalde zij haar doctoraal Geneeskunde en besloot alvorens aan haar co-assistentschap te beginnen een jaar research ervaring op te doen bij de afdeling Orthopedie van het Academisch Ziekenhuis Nijmegen (Prof Dr T J J H Slooff, Dr P Buma) Eind 1993 behaalde ze haar artsexamen cum laude Vanaf januari 1994 tot en met december 1996 was zij in de periode voorafgaande aan de opleiding Orthopedie als AGNIO onderzoeker in dienst van de afdeling Orthopedie van het Academisch Ziekenhuis Nijmegen St Radboud Alhier werd onder supervisie van Prof Dr T J J H Slooff de basis gelegd voor dit promotie-onderzoek Tijdens deze periode werd tevens de artsen opleiding voor Traditionele Chinese Geneeskunde, Auriculo-Geneeskunde en Acupunctuur Gerelateerde Technieken gevolgd bij de Nederlandse Artsen Acupunctuur Vereniging en verdiepte zij zich verder in de additieve geneeskunde bij diverse instituten in Duitsland en Zwitserland De integrale aanpak van deze vorm van geneeskunde heeft haar voorkeur gekregen en sedert april 1997 heeft zij zich gevestigd als arts voor acupunctuur en bio-informatie-therapie in Wassenaar

STELLINGEN BEHORENDE BIJ HET PROEFSCHRIFT

THE INCORPORATION PROCESS OF MORSELLIZED BONE GRAFT

Biological and Mechanical Factors

Nancy M.P. Lamerigts

I Het botverlies rond een gefaalde totale gewrichtvervanging kan klinisch alleen binnen beheersbare grenzen worden gehouden als op een geïntegreerde wijze aan de betrokken biologische, mechanische en patiëntspecifieke faktoren aandacht wordt besteed (dit proefschrift).

II De acetabulaire botmantel rond een gefaalde cup vormt een acceptabele vasculaire en vitale basis voor een revisie-reconstructie met botgraft (dit proefschrift).

III Geïmpacteerde fragmentgraft incorporeert zowel in dier-experimentele als in humaan-klinische situaties, afgezien van een verschil in tijdsinterval, volgens een identiek sequentieel patroon tot een vitale trabeculaire structuur (dit proefschrift).

IV Als gevolg van hun complexe interacties en beperkt voorspelbare gedrag moeten botinductieve substanties met terughoudendheid voor klinische doeleinden worden toegepast (dit proefschrift).

V De biomechanische belastingssituatie rond een geïmplanteerde botgraft is van invloed op de adequate omvorming van dode graft tot een vitale belastbare trabeculaire structuur (dit proefschrift).

VI De keuze voor een prothesevoorziening bij een jonge patiënt (< 55 jaar oud) moet gebaseerd zijn op feiten: een minimale follow-up van 10 jaar met meer dan 90% survival en niet op toekomstverwachtingen!

VII Ofschoon op de langere termijn voor totale gewrichtsvervangingen de spreuk "use it *and* loose it" van kracht is, kan deze voor het genezingsproces van de botgraft-revisie-reconstructie veranderd worden in "use it *or* loose it".

VIII In de negentiende eeuw zijn de grootste mijlpalen in de geneeskunde bereikt in het terugdringen van de morbiditeit en mortaliteit door het verbeteren van de levensomstandigheden; des te verwonderlijker is het dat in onze huidige stand der geneeskunde de invloed van voeding en milieu condities in het ontstaan en onderhouden van pathologie onderschat of ontkend wordt.

IX Alhoewel de "evidence based medicine" momenteel de basis is van ons westers allopathisch geneeskundig handelen, is de "Wet van Hume" nog altijd van kracht: uit feiten kan men niet zonder meer afleiden hoe men moet handelen.

X It is nice to be important, but it is more important to be nice.